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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

September 9, 2009

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held June 16 - 17, 2009 on the Evaluation of the Common Mechanism of Action of the Pyrethroid Pesticides

TO: Debbie Edwards, Ph. D.
Director
Office of Pesticide Programs

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

THRU: Laura Bailey, Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Frank Sanders, 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 June 16 – 17, 2009. This report addresses a set of scientific issues being considered by the Environmental Protection Agency pertaining to the Evaluation of the Common Mechanism of Action of the Pyrethroid Pesticides.

Attachment

cc:

Stephen Owens
James J. Jones
Betsy Shaw
Steven Bradbury
Vicki Dellarco
William Jordan
Margie Fehrenbach
Keith Matthews
Donald Brady
William Diamond
Jack Housenger
Tina Levine
Jack Fowle
Joan Harrigan-Farrelly
Lois Rossi
Richard Keigwin
Anna Lowit
Edward Scollon
Timothy Shafer
Enesta Jones
Douglas Parsons
Vanessa Vu
OPP Docket

FQPA Science Review Board Members

Steven G. Heeringa, Ph.D. (FIFRA SAP Chair)
John R. Bucher, Ph.D. (Session Chair)
Janice E. Chambers, Ph.D., D.A.B.T.
Carey N. Pope, Ph.D.
Kenneth M. Portier, Ph.D.
Daniel Schlenk, Ph.D.

FQPA Science Review Board Members

Michael E. Adams, Ph.D.
John F. Bowyer, Ph.D.
Penelope Fenner-Crisp, Ph.D.
Richard Greenwood, Ph.D.
Gaylia Jean Harry, Ph.D.
Ian R. Mellor, Ph.D.
Toshio Narahashi, D.V.M., Ph.D.
Nu-may Ruby Reed, Ph.D.
Frederic J. Seidler, Ph.D.
Sonya K. Sobrian, Ph.D.

SAP Minutes No. 2009-07

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

Evaluation of the Common Mechanism of Action of Pyrethroid Pesticides

June 16 – 17, 2009
FIFRA Scientific Advisory Panel Meeting
Held at the Environmental Protection Agency
Conference Center
Arlington, VA

Notice

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). 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 Joseph E. Bailey, SAP Designated Federal Official, via e-mail at bailey.joseph@epa.gov.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by 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

SAP Minutes No. 2009-07

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

Evaluation of the Common Mechanism of Action of Pyrethroid Pesticides

June 16 – 17, 2009
FIFRA Scientific Advisory Panel Meeting
Held at the Environmental Protection Agency
Conference Center
Arlington, VA

John R. Bucher, Ph.D., DABT
Session Chair
FIFRA Scientific Advisory Panel
Date: September 9, 2009

Joseph E. Bailey
Designated Federal Official
FIFRA Scientific Advisory Panel
Date : September 9, 2009

Federal Insecticide, Fungicide and Rodenticide Act
Scientific Advisory Panel Meeting
June 16 – 17, 2009

PARTICIPANTS

FIFRA SAP Session Chair

John R. Bucher, Ph.D., DABT, Associate Director, National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina

Designated Federal Official

Joseph E. Bailey, FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy

FIFRA Scientific Advisory Panel Members

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

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

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

FQPA Science Review Board Members

Michael E. Adams, Ph.D., Professor of Entomology & Neuroscience, Director, Interdepartmental Neuroscience Program, University of California, Riverside, California

John F. Bowyer, Ph.D., Research Pharmacologist, Division of Neurotoxicology, National Center for Toxicological Research, Department of Health & Human Services, Food & Drug Administration, Jefferson, Arkansas

Penelope Fenner-Crisp, Ph.D., DABT, Private Consultant, North Garden, Virginia

Richard Greenwood, Ph.D., Professor of Environmental Science, School of Biological Sciences, University of Portsmouth, Portsmouth, United Kingdom

Gaylia Jean Harry, Ph.D., Head of Neurotoxicology Group, Division of Intramural Research, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Ian R. Mellor, Ph.D., Research Lecturer, Division of Molecular Cell Biology and Biophysics, School of Biology, University of Nottingham, Nottingham, United Kingdom

Toshio Narahashi, D.V.M., Ph.D., John Evans Professor of Pharmacology, Department of Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

Nu-may Ruby Reed, Ph.D., DABT, Staff Toxicologist, Department of Pesticide Regulation, Health Assessment Section, Medical Toxicology Branch, California Environmental Protection Agency, Sacramento, California

Frederic J. Seidler, Ph.D., Research Professor, Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina

Sonya K. Sobrian, Ph.D., Associate Professor, Department of Pharmacology, Howard University College of Medicine, Washington, District of Columbia

INTRODUCTION

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed its review of the Evaluation of the Common Mechanism of Action of Pyrethroid Pesticides. Advance notice of the meeting was published in the *Federal Register* on March 25, 2009. The review was conducted in an open Panel meeting held in Arlington, Virginia, on June 16 - 17, 2009. Dr. John R. Bucher chaired the meeting. Joseph E. Bailey served as the Designated Federal Official.

Pyrethroids are a class of synthetic insecticides which are structurally based on the pyrethrins, botanical insecticides extracted from *Chrysanthemum cinerariaefolium*. Potential pyrethroid insecticide exposure to the general public can occur in food, water, or non-occupational settings and has increased over the past decade, due in part to a shift in usage away from the organophosphate and *N*-methyl carbamate insecticides.

The passage of the FQPA in 1996 required EPA to consider available information concerning the cumulative effects on human health resulting from aggregate exposure to multiple chemicals that have a common mechanism of toxicity. Although some uncertainties still exist, the Office of Pesticide Programs (OPP) believes that there is sufficient scientific evidence to demonstrate that the pyrethrins and synthetic pyrethroids share a common mechanism of action. The Agency's analysis and preliminary conclusions are provided in the document titled: "Draft Science Policy Paper: Common Mechanism Grouping (CMG) for the Pyrethrins and Synthetic Pyrethroid Pesticides." This draft issue paper was developed by the Health Effects Division (HED) of OPP with support from EPA's Office of Research and Development (ORD). Specifically, OPP is proposing that the naturally occurring pyrethrins and synthetic pyrethroids form a common mechanism grouping based on 1) shared structural characteristics; and 2) shared ability to interact with voltage-gated sodium channels (VGSC), resulting in disruption of membrane excitability in the nervous system, and ultimately neurotoxicity characterized by two different toxicity syndromes. OPP is further proposing to divide the pyrethroid CMG into two subgroups representing Type I and II pyrethroids based on differences in structure, sodium channel perturbations, and neurobehavioral effects.

The Agency has solicited comments from the Panel on science issues related to the common toxicity pathway for the pyrethroid insecticides, remaining uncertainties, and the proposal to separate the pyrethroids into two subgroups (Type I and Type II).

Steven Bradbury, Ph.D., (Deputy Office Director for Programs, Office of Pesticide Programs) and Tina Levine, Ph.D., (Director, Health Effects Division, Office of Pesticide Programs) provided opening remarks at the meeting. The agenda for the meeting included presentations by Anna Lowit, Ph.D. and Edward Scollon, Ph.D. (Health Effects Division, Office of Pesticide Programs) and Timothy Shafer, Ph.D. (Integrated Systems Toxicology Division, Office of Research and Development) as well as public comments.

PUBLIC COMMENTS

Oral statements were presented by:

Dana Sargent, Charles Breckenridge, Dan Minnema, and John Clark on behalf of the Pyrethroid Working Group

John Clark on behalf of Janet Hemingway, Director of the Liverpool School of Tropical Medicine and CEO of the Bill and Melinda Gates funded Innovative Vector Control Consortium.

Written Statements were provided by:

Dana Sargent on behalf of the Pyrethroid Working Group

SUMMARY OF PANEL DISCUSSIONS AND RECOMMENDATIONS

EPA is proposing that naturally occurring pyrethrins and synthetic pyrethroids be considered to form a common mechanism group based on their shared ability to interact with voltage-gated sodium channels (VGSCs) which causes disruption of nervous system membrane excitability resulting in neurotoxicity syndromes. The Panel agreed with the Agency that pyrethroid insecticides share the VGSC as a common molecular target site, increasing its open time. The Panel noted that all pyrethroids tested thus far do act on the VGSCs with both Type I and Type II pyrethroids increasing the open time of the VGSC as a primary target. However, the nature and extent of this effect is different for the two types. Most Panel members agreed with the suggestion that the pyrethroids should be grouped into the same common mechanism group based on their clear effects on VGSCs. However, at least two Panel members were not convinced that the Type I and Type II pyrethroids should be grouped in the same common mechanism group due to the different physiological effects and toxicity symptoms.

Regarding uncertainty associated with the lack of an *in vivo* biomarker for sodium channel effects, the Panel acknowledged that identification of such a biomarker would be difficult. In any case, Panel members did not believe that a biomarker was necessary for the conduct of a cumulative risk assessment and the absence of one does not exclude the common mechanism group consideration. At this time, they believed that evaluation of the compounds should proceed without a biomarker.

The Agency concluded that the current body of evidence on pyrethroid interaction with other ion channels, namely calcium and chloride channels and ligand-gated chloride channels, does not support the characterization of these interactions as a common key event in pyrethroid toxicity. The Panel noted that published literature shows that voltage-gated calcium channels are affected by Type II, but not Type I pyrethroids. Thus, some Panel members believed that a potential role of calcium channels as a major molecular target site for Type II compounds could not be dismissed. However, knowledge of chloride channels as primary targets of Type II pyrethroids remains rudimentary compared to calcium and sodium channels and it is difficult to attribute chloride channel modification specifically to Type II pyrethroid poisoning toxicity. The presence of the α-cyano substituent in Type II pyrethroids leads to a broader range of ion channel targets, different symptoms, and potentially different mechanisms of toxicity. However, at this time, the lack of knowledge makes it impossible to associate the effects of Type II pyrethroids on calcium channel modification to symptoms and toxicity. Therefore, the Panel suggested that the effects of Type II pyrethroids on calcium and chloride channels could not be used as evidence against placing them into the same common mechanism group.

The Panel was asked to comment on the scientific support for and against the Agency's conclusion that the heterogeneity among α- and β-subunit combinations comprising sodium channels does not discount the role of sodium channel interaction as a critical and initial key event in pyrethroid toxicity. The Panel agreed that, while heterogeneity in channel properties contributes to pharmacodynamic variability, the

interaction of pyrethroids with the sodium channel is a key event in the neuronal toxicity of pyrethroids. Incomplete knowledge of the role of α- and β-subunits does not discount this key event.

The Agency proposed to divide the pyrethroids into two subgroups, Type I and Type II, based on structural differences related to the absence or presence of an α-cyano group and two distinct toxicity syndromes. The Panel noted that while the purpose or use of defining two sub-categories of pyrethroids was unclear, there was good evidence for a separation based on the absence or presence of an α-cyano group into Type I and Type II subgroups, respectively. However, there are instances where structural differences do not predict a clear outcome or the effects are mixed. For example, it was pointed out by several panel members that there is overlap in behavioral toxicity produced by Type I and Type II pyrethroids. The Panel believed that while the concept of subgroups is scientifically sound, the actual basis for these subgroups and the endpoints to distinguish between the subgroups remain in question. The Panel advised caution in relying on any one endpoint for determining subtypes or in defining low dose effects at this time. The Agency's structural approach to assign pyrethroids to Type I and Type II subgroups is logical, but as noted earlier, there are cases where structure does not predict outcome or mixed effects are observed. Thus, there is a risk of incorrectly assigning a compound to a subgroup. The Panel believed that the basis for assignment must be more than structure alone. They recommended that those chemicals which exhibit both Type I and Type II pyrethroid characteristics should either be included in both subgroups or in a separate third subgroup. However, the majority of the Panel concurred that keeping a single common mechanism group might be the simplest option for cumulative risk assessment purposes. At least one Panel member suggested that two separate cumulative assessment groups (CAGs) might be more appropriate, one including all of the pyrethroids meeting the Type I criteria and the other made up of all the pyrethroids meeting the Type II criteria. Those substances with mixed characteristics would be included in both groups.

The Panel agreed with the Agency's conclusion that the limited number of mixture studies pre-dating Wolansky et al. (2009) do not allow for the determination of the nature of interaction(s) between members of the pyrethroid class, following co-exposures. These results cannot be used to determine the nature of interaction for application in a cumulative risk assessment. The Panel noted that some of the neurobehavioral endpoints measured in the Neurotoxicity Screening Battery guideline study (i.e., motor activity and elements of the functional observation battery) may be of value in further determining the nature of pyrethroid mixture interactions. However, existing data on motor activity observed following exposure to substances of each type do not show a distinct pattern of type specificity. The predictive value of the enhanced Functional Observational Battery needs to be evaluated. The Panel provided suggestions about additional research that could be undertaken on mixture interactions that could build on previous studies conducted and referenced in EPA's proposal (e.g., Crofton and Reiter, 1984; 1988; Wolansky et al., 2006; 2009; Nemec, 2006). In summary, the Panel recommended that at least one additional *in vivo* mixture study be conducted testing chemicals in pairs or small sets, with acoustic startle seen as a good endpoint for evaluation.

PANEL DELIBERATIONS AND RESPONSE TO CHARGE

Charge Question 1 - *Common Pathway to Neurotoxicity*

a. OPP is proposing that the naturally occurring and synthetic pyrethroids share the ability to interact with voltage-gated sodium channels (VGSC) resulting in disruption of membrane excitability in the nervous system, and ultimately neurotoxicity. The shared ability provides the initial and common key event in the pathway to pyrethroid neurotoxicity and thus provides a basis for forming a common mechanism group. As described in Section 4.0 of the draft paper, the Agency has determined that interaction with the VGSC is an initial and common key event in the pathway to pyrethroid neurotoxicity.

Unlike the cholinesterase inhibiting organophosphorus and *N*-methyl carbamate pesticides, pyrethroids lack a readily measurable *in vivo* biomarker for the initial key event (i.e., sodium channel interaction). Despite this, the scientific evidence correlating pyrethroid-induced changes in VGSC function with neurotoxicity for purposes of forming a common mechanism grouping is substantial. Given the availability of extensive studies on the mechanism of toxicity and toxic effects of pyrethroids, the lack of an *in vivo* biomarker does not preclude grouping via a common mechanism.

Please comment on the evidence which does and does not support the Agency's proposal that sodium channel interaction provides the initial and common toxic event in the pathway to neurotoxicity for the synthetic pyrethroids and pyrethrins. As part of your response, please comment on the uncertainty associated with lack of a readily measurable *in vivo* biomarker for sodium channel interaction.

Panel Response – The Panel acknowledged the efforts EPA has taken to propose that the pyrethroid insecticides form a single common mechanism grouping. The proposal by EPA is based on extensive investigations of data in the literature and is, to a large extent, successful in defining the issue.

The Panel agreed with the Agency that pyrethroid insecticides share the voltage-gated sodium channel (VGSC) as a common molecular target site, increasing its open time. Naturally occurring and synthetic pyrethroids share the ability to interact with VGSCs resulting in disruption of membrane excitability in the nervous system. A number of *in vitro* studies have clearly established that VGSCs are a major target site of pyrethroids. It is important to note that all pyrethroids thus far tested act on the VGSCs. Both Type I and Type II pyrethroids prolong opening of the VGSC as a primary target, although the nature and extent of this effect is different between them. Repetitive firing in neurons is consistent with the action of Type I pyrethroids on the VGSC and little or no depolarization is a consequence of moderate VGSC modification leading to moderate increases of VGSC open time. Furthermore, no hyperpolarizing shift of voltage-dependent activation and no major long-term membrane depolarization occur following exposure to Type I pyrethroids. Symptoms associated with Type I pyrethroid poisoning

are referred to as Tremor (T)-syndrome. The affinity of VGSCs for Type II pyrethroids is apparently increased with the addition of the α-cyano group and this modification may also broaden the range of their targets to include other voltage-gated ion channels (see Charge 1b). Type II pyrethroids prolong VGSC openings from the normal few milliseconds to seconds or even minutes and shift voltage-dependent activation to more negative potentials, leading to opening of VGSCs close to the resting potential. Long-term depolarization results from these actions. Type II pyrethroids cause much longer openings than do Type I pyrethroids and this is associated with a distinctive set of poisoning symptoms referred to as Choreoathetosis/Salivation (CS)-syndrome.

Modification of only 1% or less of the VGSC population present in the nerve membrane is sufficient to cause disruption of neuronal activity. This "toxicity amplification" results when a chemical acts on the target site via a threshold phenomenon. In the case of Type I pyrethroids, once elevation of the depolarizing after-potential (which follows the action potential as a result of the prolongation of sodium current) reaches the threshold for excitation, repetitive discharges are evoked which cause hyper-excitation in poisoned animals.

The Panel agreed that the VGSC modulation is an important key event in the "common mechanism" from which various whole body symptoms result. However, the question of exactly how pyrethroid-induced VGSC modulation is related to *in vivo* symptoms of poisoning remains largely unanswered, although it is somewhat better understood for Type I pyrethroids than for Type II pyrethroids. Pyrethroids certainly affect other target sites (e.g., modulation of calcium channels by Type II pyrethroids) and this could contribute to observed poisoning syndromes, though the mechanisms involved are not well understood. Actions on targets other than the VGSC may or may not be related to the toxic action of Type II pyrethroids. Even though the two types of compounds are working on the same channel, their effects are quantitatively and qualitatively different, especially at high doses. Extending *in vitro* data on voltage-gated channel alterations to the symptoms of poisoning in whole animals or humans is a daunting task.

The Panel raised a number of issues for consideration by the Agency in these deliberations. Many were related to the integrated nature of the nervous system and the interdependence of its component parts. It is extremely important to recognize that any chemical (with the exception of some natural toxins, such as tetrodotoxin) can interact with multiple target sites. Because there is evidence that Type II pyrethroids affect other ion channels (e.g., voltage-gated calcium channels which are discussed in Charge 1b), it is unwise to assume that all pyrethroids act through a single ion channel target to produce neurotoxicity. While VGSC interaction is a common mechanism for all pyrethroids, depending on the physicochemical properties of the insecticide, the nature of the interaction with a given target and the range of target sites affected may vary. This is supported by the fact that Type I and Type II pyrethroids cause distinctly different physiological effects on neurons (excitation vs. block) and two different poisoning syndromes (T-syndrome and CS-syndrome). At least two Panel members were not convinced that the Type I and Type II pyrethroids should be placed in the same common

mechanism group due to the physiological and toxicity differences between the two types.

The Panel reiterated to the Agency that it is of critical importance to realize that any modification of the functioning of VGSCs will have a vast influence on many neuronal systems. For example, prolongation of sodium currents by pyrethroids will cause not only neuronal hyperactivity producing repetitive action potentials, but also an increase in the release of various transmitters through a cascade of synaptic events in the brain which contains billions of neurons and synapses. The final neurobehavioral outcome could be hyperactivity or hypoactivity resulting in T-syndrome and CS-syndrome, eventually leading to paralysis. It is extremely important to consider the role of synaptic networks in the brain when identifying a specific target of neurotoxicity. For example, most pyrethroids have been shown to induce calcium influx and glutamate release. It is very difficult to relate these actions directly to either the T- or CS-syndrome because the released glutamate will stimulate synaptic and extrasynaptic glutamate receptors and/or cause neuronal damage (excitotoxicity) depending on the amount of glutamate released. The effects of glutamate involve not only neurons, but also depend upon its interactions with glial cells (Lopez-Redondo et al., 2000). The latter can have multiple and diverse effects leading to exacerbation or attenuation of neuronal damage (Watanabe et al., 2000; Block and Hong, 2005, Taylor et al., 2005; Garden and Möller, 2006, Davalos et al., 2005; Barger et al., 2007).

The Panel discussed unique properties of the pyrethroid types, such as their temperature dependent effects. Type I pyrethroids exhibit a negative temperature dependence of toxicity or killing potency; i.e., lowering the temperature increases the toxicity in insects. However, data on Type II pyrethroids are somewhat controversial and any temperature dependency remains unclear. The temperature dependency of pyrethroid-induced VGSC modification remains important with regard to mechanism of action even though mammals maintain their body temperature at 37°C. A question then is raised as to whether pyrethroid actions on other ion channels show temperature dependence and, if not, whether these actions at non-VGSCs can be regarded as target sites producing the symptoms of poisoning *in vivo*.

The Panel discussed the fact that the VGSC can serve as the target for many chemicals other than pyrethroids. As an example, Type I pyrethroids and dichlorodiphenyltrichloroethane (DDT) exert almost the same mechanism of action on the VGSC (Usherwood et al., 2005; Davies et al., 2007; Narahashi and Haas, 1968, Lund and Narahashi, 1981; Song et al., 1996). Furthermore, *in vitro,* the potency of both pyrethroids and DDT is temperature dependent, increasing as the temperature is lowered. Also there is cross resistance between pyrethroids and DDT in insects, suggesting a common or overlapping binding site and/or mechanism of action (O'Reilly et al., 2006; Usherwood et al., 2007). These observations are not compatible with a common toxophore limited to the pyrethroid molecules. The Pyrethroid Working Group (PWG) contends that, contrary to EPA's claim that all pyrethroids share a common toxophore, the toxicity of all pyrethroids is related to the entire molecule. Since pyrethroids act on multiple target sites, it is possible they have multiple toxophores that bind to the

respective target sites. However, since limited experimental evidence has been accumulated to date, definitive conclusions regarding the issue of pyrethroid toxophores are not possible at the present time. Data obtained from other chemicals that target VGSCs and that extend the range of physicochemical and toxicological properties in the data set may possibly help advance the understanding of the Type I and Type II domains of activity.

Significant differences between rats and humans in the sensitivity of the target site to chemicals in general have been reported in the literature. Based on the currently available data, it would be premature to draw conclusions concerning the pharmacodynamic issues as raised by the PWG. However, it is very important to acknowledge that differences in the sensitivity to various pyrethroid chemicals between the rat and human may exist.

The Panel was in agreement that the absence of an *in vivo* biomarker of toxicity does not exclude the common mechanism group consideration. The observation that pyrethroid resistant insects have mutations in their VGSCs (and not in any other channels) is a clear *in vivo* indication that the VGSC is the primary target in insects. However, the Panel reached no conclusion as to whether there is or could be a reliable *in vivo* biomarker for pyrethroid action on mammalian VGSCs. Unlike the organophosphate and the N-methyl carbamate insecticides, for which blood cholinesterase can be measured easily, no easy methods are available for the pyrethroids. The requirement of non-invasive methods for a marker should be considered. The Panel was in agreement that while a distinct biomarker is not necessary for the conduct of a cumulative risk assessment, considerations of metabolism and detoxication are important, especially regarding the assessment of individual susceptibility that depends on both pharmacodynamic and pharmacokinetic activity.

b. The Agency is aware of studies which show that pyrethroids can bind to other sites such as the calcium and chloride ion channels and ligand-gated chloride channel currents. The Agency acknowledges that interaction between the pyrethroids and these sites may mediate their potency. However, the data which support interactions with the calcium, chloride, and ligand-gated chloride channels are not sufficiently robust for purposes of common mechanism grouping under the FQPA. Therefore, these pathways do not provide the basis for establishing their binding as a common key event leading to neurotoxicity. The Agency has concluded that the evidence on pyrethroid interaction with the calcium and chloride channels and ligand-gated chloride channels is limited and inconsistent. The Agency has therefore concluded that the evidence does not support characterizing these interactions as a common key event in the pathway to neurotoxicity by the pyrethrins and synthetic pyrethroids (Section 4.2.5).

Please comment on the evidence which does and does not support this determination.

Panel Response - The development of deltamethrin and other α-cyano-pyrethroids fundamentally changed the manner in which the toxicology of synthetic pyrethroids is evaluated. Studies have led to the classification of pyrethroids into Type I (non-α-cyano) and Type II (α-cyano) categories. Generally speaking, Types I and II pyrethroids modify sodium channels by slowing transitions between different channel states (closed→open→inactivated→closed). Type II pyrethroids, by virtue of the α-cyano group, are much more potent and efficacious modifiers of sodium channels than are the Type I compounds because they cause much longer open times and shift voltage-dependent activation of sodium channels to more negative potentials. A key physiological consequence of sodium channel modification by Type II pyrethroids is prolonged opening of the sodium channel causing depolarization of neurons, block of impulse conduction, and a set of symptoms distinct from those of Type I pyrethroids. Type I pyrethroids, in contrast, produce relatively shorter prolongations of channel openings, raising the depolarizing after potential to the threshold level for generating action potentials and hence, cause repetitive discharges in neurons leading to prolonged excitation and symptoms associated with T-syndrome. Sodium channel modification is undoubtedly a key event in the toxicity for all pyrethroids, but differences in channel modification, physiological actions, and the signs and symptoms of toxicity may suggest somewhat different mechanisms of toxicity for Type I and Type II pyrethroids.

Differences in the properties of Type II pyrethroids appear to broaden their ion channel specificity. Although EPA's Draft Science Policy Paper characterizes recent studies on their effects on calcium and chloride channels as "not sufficiently robust", "limited", and "inconsistent", some Panel members believed that a potential role for calcium channels as a major molecular target site of Type II pyrethroids cannot be dismissed.

A series of papers published in the peer-reviewed literature has shown that voltage-gated calcium channels (VGCC) in nerve terminals isolated from the mammalian brain (synaptosomes) are affected by Type II, but not Type I pyrethroids (Symington et al., 2007a). These are robust effects observed at concentrations in the sub-picomolar range, similar to concentrations that modify sodium channels. They include increased calcium flux into synaptosomes and increased neurotransmitter release. The effects are observed in the presence of tetrodotoxin, which is thought to eliminate the contribution of sodium channel modification to the observed effects. Additional studies show that picomolar concentrations of Type II pyrethroids modify the N-type ($Ca_v2.2$) calcium channel expressed heterologously in frog oocytes (Symington et al., 2007b). While this modification depends on the phosphorylation state of the calcium channel, the effects resemble those observed for sodium channels, including increased peak inward current, slowing of inactivation, and a shift of voltage-dependent activation to more negative potentials. Increased calcium influx would be consistent with CS-syndrome toxicity signs and symptoms. Furthermore, this modification is observed at sub-nanomolar concentrations and is observed only when using the same enantiomeric configuration of pyrethroids active on sodium channels. One difference between the ways that pyrethroids modify VGSCs and VGCC is the absence of persistent tail currents in the latter, and these are a hallmark of sodium channel modification by Type II pyrethroids. However, this is

not viewed as weakening the argument for the relevance of calcium channel modification in the Type II poisoning syndrome.

Somewhat less convincing is the argument for chloride channels as primary targets of Type II pyrethroids. Knowledge of chloride channels remains rudimentary compared with that of sodium and calcium channels. Block of peripheral and central voltage-gated chloride channels by α-cyanopyrethroids (e.g., deltamethrin and cypermethrin, but not cismethrin) is consistent with CS-syndrome signs and symptoms caused by Type II pyrethroids. Single channel recordings of chloride currents (probably the maxi chloride channel) show significant reductions in open channel probability (Po) with Type II pyrethroids, but these changes are small when compared with data with $Na_V1.2$ (one of the less sensitive Na_Vs) that shows much larger increases (1 – 2 orders of magnitude) in the probability of opening (Peng et al., 2009). However, not all α-cyanopyrethroids (e.g., esfenvalerate and λ-cyhalothrin) affect chloride channels in the same fashion and some non-α-cyanopyrethroids (e.g., bioallethrin) are effective blockers (Burr and Ray, 2004). Therefore, it is difficult to attribute chloride channel modification specifically to the Type II pyrethroid poisoning syndrome. Furthermore, the concentrations of pyrethroids needed for modification of chloride channels (10 micromolar) are relatively high. Voltage-gated chloride channels are also expressed in glia and it is known that glial dysfunction can lead to altered calcium and glutamate levels in nervous tissue, resulting in toxicity and seizures. Thus, pyrethroid interactions with chloride channels in neurons and glia could be involved in the neurotoxic process, but the data are insufficient to conclude that voltage-gated chloride channels are the primary targets of pyrethroids. Additionally, data on $GABA_A$-chloride channels indicate very weak effects of pyrethroids and can probably also be discounted (Orgata et al., 1988). The Panel agreed with the Agency's conclusion that the data on chloride channels are not robust, are limited, and in general, inconsistent with a role in pyrethroid-induced toxicity.

In summary, the Panel agreed with the suggestion that sodium channels are a common molecular target of all pyrethroids, but that voltage-dependent calcium channels also must be considered as one of the primary targets of the Type II pyrethroids. The presence of the α-cyano substituent in Type II pyrethroids leads to a broader range of ion channel targets, different symptoms, and potentially different mechanisms of toxicity. However, the dearth of knowledge relating ion channel modification to symptoms and toxicity makes it impossible to attribute effects on calcium channels to toxicity at this time. Therefore, the Panel suggested that the effects of Type II pyrethroids on calcium and chloride channels cannot be used as evidence against placing them into the same common mechanism group.

Charge Question 2 - Sodium channel structural heterogeneity

Briefly, mammalian sodium channels are comprised of α and β subunits that exist in multiple isoforms, giving rise to tissue, regional and lifestage heterogeneity in sodium channel expression (Goldin 2001; Plummer and Meisler 1999). Mammalian neurons typically express multiple isoforms of both α and β subunits, making it difficult to determine the composition of subunits comprising sodium channel

currents in native neurons. Evaluation of specific alpha- and beta-subunits (either alone or in combination) may be interesting for purposes of evaluating species differences, potential population pharmacodynamic variability, and lifestage differences. With respect to the proposal to form a CMG, however, incomplete knowledge of the role of the α and β subunits in pyrethroid toxicity does not discount the role of sodium channel interaction as a key event in pyrethroid toxicity. As described in Sections 4.2 and 5.0, the Agency has concluded that although there is heterogeneity among the subunit combinations, the pathway of toxicity remains the same---namely that sodium channel interaction is a critical and initial key event in toxicity of pyrethroids.

Please comment on the scientific support for and against the Agency's conclusions with respect to the sodium channel structural heterogeneity information.

Panel Response - It is clear from the literature that a wide range of variants of the VGSC occur in mammals and insects (the two groups of animals in which most of the studies of this system have been effected), and that the variation is achieved in different ways in the two classes of organisms. Many of the toxicological responses of insects in the early stages of pyrethroid poisoning are due to the effects on the sensory systems and the effects of disruption of the central nervous system become apparent later in the course of poisoning. Overall the sensitivity of insect VGSCs to pyrethroids may be 1000 times greater than that of mammalian VGSCs. In mammals the distribution of structural and functional variants differs between different regions of the nervous system, and in the same region with stage of development (Thun et al., 2009). The range of variation due to the mixture of channels present could be further broadened *in vivo* through modulatory effects.

The available information, much of it obtained from expression studies in *Xenopus* oocytes, indicates that there is a very wide range of combinations of subunits that can provide functional VGSCs with different properties. Much work has been published in the peer reviewed literature providing evidence that a range of amino acid substitutions in individual subunits can produce changes in channel properties, some of which are associated with disease syndromes (Yu and Catterall, 2003; Goldin, 2001), and some of which are differentially susceptible to Type I and Type II pyrethroids. Studies in insects have shown that sensitivity to pyrethroids is increased with variants showing increased persistent current. In mammals, these sorts of changes in the activation and inactivation kinetics are modified by the presence or absence of different β subunits (Isom, 2001). Some mutations in the VGSC in humans are associated with epilepsy. The mutated channels show only a very small (a few percent) increase in persistent current, which is sufficient to cause gross pathological symptoms.

It is not straightforward to extrapolate from *in vitro* systems to the *in vivo* situation where the lipid environment will be different from that in oocytes, and modulatory mechanisms may be operating. Caution needs to be applied since, for instance, in oocytes, human $Na_v1.8$ is more sensitive to pyrethroid exposure than human Na_v 1.2, but in intact brain, $Na_v1.2$ is more important, and $Na_v1.8$ is relatively less

important. Moreover, the *in vivo* system contains a range of variants, and small changes (a few percent of the total population) can affect the properties of neurons. One problem when extrapolating from experiments in oocytes to humans is a lack of knowledge of the range of VGSC phenotypes (corresponding to the genotypic variation) that is present in human populations, and how the different combinations of subunits in various regions of the central nervous system respond to pyrethroids. Single mutations in mammalian VGSCs can produce large changes in sensitivity to pyrethroids; for instance a single amino acid mutation (I874M) in rat $Na_v1.2$ increases sensitivity to levels found in insect channels (Peng et al., 2009; Vais et al., 2000).

Although there is uncertainty concerning the exact nature of the interaction between the pyrethroids and the VGSC, and the *in vivo* behavior of the different variants of the channel protein, this does not affect the assumption that the VGSC is a common, primary target site for pyrethroid insecticides. The toxicological evidence available in the peer reviewed literature strongly supports the idea that disruption of the VGSC is an important primary lesion associated with the complex set of secondary lesions observed *in vivo* following exposure to Type I pyrethroids. Interaction of Type II pyrethroids with VGSC causes major changes in the channel properties and major disruption of neuronal function. However, the linkage between changes in neuronal properties associated with the primary lesion and the gross symptoms of poisoning used for diagnostic purposes is less clear for Type II pyrethroids than for Type I pyrethroids.

The Panel members agreed with the Agency's statement that the interaction of pyrethroids with the sodium channel is a key event in the neuronal toxicity of pyrethroids. It was agreed that the heterogeneity in channel properties should be considered as a factor that contributes to variation between species and between developmental and physiological states within a species in risk assessments. However, while the *in vitro* studies of channel properties provide valuable insights into the structure and function of the VGSC, and will ultimately help to demonstrate the way in which pyrethroids interact with the VGSC, the uncertainty involved in extrapolating from *in vitro* observations to *in vivo* behavior of VGSCs is large. In the current context, the detail may well divert attention from the task of risk assessment where *in vivo* information is more relevant.

Summary

- Heterogeneity in channel properties contributes to pharmacodynamic variability
- The interaction of pyrethroids with the sodium channel is a key event in the neuronal toxicity of pyrethroids
- Incomplete knowledge of the role of the α and β subunits in pyrethroid toxicity does not discount the role of sodium channel interaction as a key event in pyrethroid neuronal toxicity

Charge Question 3 - Sub-grouping the Type I and II pyrethroids

a. The Agency has proposed to separate the pyrethrins and synthetic pyrethroids into Type I and Type II subgroups as discussed in detail in Section 5.0 of the Draft Science Policy Paper. Briefly, this proposal is based on the structural difference in Type I and Type II pyrethroids, i.e, the absence or presence of an α-cyano group, respectively[1]. This structural difference is correlated with length of time the sodium channel is inactivated (-CN=shorter; +CN=longer) which in turn corresponds with the 2 distinct toxicity syndromes (-CN=T syndrome; +CN=CS syndrome). This separation is based on a weight of the evidence evaluation that considered both historical and newer studies from *in vitro* (i.e., intact and transected sodium channels and microelectrode array) and *in vivo* studies (i.e., motor activity and functional observational battery).

Please comment on the evidence which does and does not support this determination.

Panel Response - While the purpose of defining two sub-categories of pyrethroids was unclear (the EPA Guidance on Common Mechanism does not describe a use for subgroups within a common mechanism group), there is good evidence to support a separation into Type I and Type II subgroups based on the absence or presence of an α-cyano group, respectively. The absence or presence of an α-cyano group is a good predictor of the compound's effects at the VGSCs and VGCCs. However, there are instances where the structural differences do not predict the outcome (e.g., the effect of permethrin or fenpropathrin on VGCCs) or the effects are mixed.

The *in vivo* evidence to support classification into Type I and Type II is less apparent. Although the sub-categories can be clearly distinguished by some effects (e.g. increase vs. decrease in body temperature), they also have common effects. The first pyrethroid Functional Observational Battery (FOB) study (McDaniel and Moser, 1993) described striking differences between the "CS" and "T" syndromes (at high doses). However, the study also described similarities between the two classes, particularly with regard to neuromuscular variables which include grip strength and gait abnormalities for both types of pyrethroids (McDaniels and Moser, 1993). A similar pattern is found in the group data from the PWG FOB studies (Nemec 2005 and 2006) which also indicated that there is overlap in the behavioral responses. Clonic convulsions, abnormal gait and resistance to removal and handling were responses seen in rats given both Type I and Type II compounds.

The motor activity profiles for all pyrethroids show altered levels of activity relative to controls. Although the direction of the change (i.e., decrease or increase) is a function of the species tested and the route of administration, in general, pyrethroids decrease motor activity. Motor activity can be impaired by a number of specific as well as non-specific factors. The motor activity profiles do not show significantly different patterns between the two categories to be able to use them to assign chemicals to one or the other subgroup of pyrethroids.

In contrast, the data sets resulting from the application of the enhanced FOB study do provide for the separation and identification of some distinct profiles of behavior that distinguish between the two subgroups of pyrethroids. Nevertheless, the analysis was only descriptive (used to generate a pattern profile) and lacked a hypothesis. Furthermore, at low doses, the distinction between subtypes is not clear since under these conditions all of the tested compounds have similar effects. It will be possible to have greater confidence in the model based on the FOB if data from new compounds is used to test the hypothesis that the agent's FOB subgroup cluster can be predicted on the basis of chemical structure. Addition of other quantitative endpoints, such as startle reactivity, may help to distinguish between the Type I and Type II pyrethroids. The Panel anticipates that the results from EPA's ongoing microelectrode array (MEA) studies will be extremely helpful.

While the concept of subgroups is scientifically sound, the actual basis for these subgroups and the endpoints that can be used to distinguish between them remain in question. There are both structural and electrophysiological data that support dividing pyrethrins into Type I and Type II subgroups. However, the data for these two variables are not clean cut and are presented with several caveats in EPA's Draft Science Policy Paper. Behavioral data in rats at low doses is equivocal with respect to the dichotomy. The Panel, therefore, advised caution in relying on any one endpoint for determination of subtypes or in defining low dose effects at this time. The differences between Type I and Type II pyrethroids identified thus far are interesting and might be a key to elucidating the mechanisms for neurotoxicity.

b. With respect to assigning the pyrethroids to sub-groups;

- **The Agency's preliminary designation for 11 pyrethroids and pyrethrin is based on a weight of the evidence assessment utilizing three key lines of evidence: presence/absence of the alpha-cyano group, effects on sodium channel kinetics, and *in vivo* toxicity syndromes.**
- **Five additional pyrethroids are being characterized in a special FOB study. For these five the structure also is known. Thus for tetramethrin, cyphenothrin, imiprothrin, phenothrin, and prallethrin, information from two lines of evidence will be available (structure, toxicity syndrome) for assigning these.**
- **Tralomethrin is metabolized to deltamethrin *in vivo* and is also converted in the environment to deltamethrin. As such, given the presence of the alpha-cyano group and its relationship to deltamethrin, the Agency expects tralomethrin to be assigned a designation of Type II.**
- **Cinerin and jasmolin are naturally occurring pyrethrins and do not have the alpha-cyano group on their structure. Thus, the Agency expects cinerin and jasmolin to be assigned a designation of Type I.**
- **Two other pyrethroids, metofluthrin (non-cyano) and fluvalinate (cyano) have scant databases. With respect to their toxicity, the Agency is unaware of detailed characterization of their profiles which would allow designation. Moreover, the Agency is unaware of studies describing their interactions**

with sodium channels. The Agency expects metofluthrin and fluvalinate to be designated as Type I and Type II compounds, respectively, based on structure.

Please comment on the Agency's approach to assigning the pyrethroids to the Type I and Type II sub-groups. Please include in your comments consideration for those without special FOB information and for which structure will be the major determinant in their designation.

Panel Response - The Agency's approach to assigning the pyrethroids to the Type I and Type II sub-groups is logical. However, while structure is useful in predicting the physiological and behavioral outcomes in many cases, there are compounds for which structure fails to predict outcomes or that display both Type I and Type II characteristics. Thus, with incomplete information, there is the possibility of incorrectly assigning a compound to a subgroup. The Panel believed that it is critical to have more information than structure alone. It would be useful to have sodium channel data, and perhaps, calcium channel data to assist in the categorization of these compounds.

With respect to the third bullet in the Agency's conclusions above, if tralomethrin is readily converted to deltamethrin environmentally or metabolically, then the categorization of tralomethrin as deltamethrin-like seems reasonable.

c. Two pyrethroids, fenpropathrin and esfenvalerate, exhibit characteristics of Type I and Type II compounds (i.e., "mixed" Type). In the anticipated cumulative risk assessment, the Agency must determine the appropriate approach for these two. In performing exposure assessment and ultimately in estimating human risk, several options have been identified—include fenpropathrin and esfenvalerate in the Type I subgroup, in the Type II subgroup, or in both subgroups.

Please comment on these possible options and any others identified by the Panel.

Panel Response – The Panel was unclear on the Agency's intended use for the subgroup categorization. Further, it is not obvious what needs to be done in the case of the subgroup of compounds that do not fit readily into either Type I or Type II pyrethroids. The Panel believed it is appropriate to include compounds that exhibit some characteristics of both subgroups into both Type I and Type II categories. Alternatively, a third subgroup (mixed types) could be established. Of course, once all endpoints of effect are systematically evaluated, other combinations of "mixed" compounds may result. If this occurs, having a single common mechanism group might be the simplest option for the purposes of cumulative risk assessment. At least one Panel member suggested that two separate cumulative assessment groups (CAGs) might be more appropriate, one including all of the pyrethroids meeting the Type I criteria and the other made up of all the pyrethroids meeting the Type II criteria. Those substances with mixed characteristics would be included in both groups.

A Panel member also indicated that it was not clear which dependent variables were being used to classify fenpropathrin and esfenvalerate as 'mixed' type pyrethrins. Table 1 in the PWG study (PWG-TOX-2007-01) indicated that for behavioral (FOB) measures, only fenpropathrin was classified as mixed, as does Table 2 in the EPA Draft Science Policy Paper.

Charge Question 4 - Evaluation of Dose-Addition of pyrethroids

As discussed in Section 4.4, there are a limited number of mixture studies on pyrethroids. Electrophysiological studies have evaluated mixtures of two pyrethroids but used excessive doses and/or lack robust study designs and statistical analyses. As such, these studies preclude thorough evaluation of dose or effect addition. More recent studies include Wolansky et al. (2009) and a study currently underway at EPA which evaluate motor activity *in vivo* and microelectrode arrays, an assay for mammalian neural networks, *in vitro*. These studies were specifically designed to test dose additivity but endpoints measured in both studies lack in their ability to establish dose additivity specifically at the level of the sodium channel.

Please comment on additional research which could be undertaken to evaluate the assumption of dose-addition as it relates to the proposed common mechanism pathway.

Panel Response - The Panel agreed with the Agency's conclusion that the limited number of mixture studies pre-dating Wolansky et al. (2009) does not allow for the determination of the nature of interaction(s) (e.g., additive, synergistic, antagonistic) between members of the pyrethroid class, following co-exposure in pairs or more. Thus, their results cannot be used to determine the nature of interaction and could not be used in the development of a cumulative risk assessment.

Several different endpoints are measured in the Neurotoxicity Screening Battery guideline study (OPPTS 870.6200). This study is required for all food-use and non-food use conventional chemicals which include the pyrethroid class. The endpoints measured in this study should be evaluated as potential candidates for determining the nature of mixture interactions at relevant doses. These endpoints fall into the following categories 1) Motor activity; 2) Elements of the Functional Observational Battery; and 3) Neuropathology. However, the Panel overall raised caution with regard to determining a critical effect and a peak time to effect for any such additivity assessment. A few Panel members questioned the level of sensitivity of the Neurotoxicity Screening Battery to detect the nature of the interaction between pyrethroid compounds.

Motor Activity: EPA's Draft Science Policy Paper describes existing non-registrant generated data and concludes that a decrease in motor activity is observed with both Type I and Type II pyrethroids and that this does not show a pattern that can be used to separate the two classes. On the basis of this common effect, it was proposed that motor activity could serve as a basis for establishing the nature of interactions between compounds applied in mixtures, even though, as noted, a full understanding of the

mechanism by which pyrethroids produce this decrease does not exist at this time. Given the differences in methods and apparatus that have been used to measure motor activity, it is recommended that any new data should be collected with the same study design in order to maximize data comparability. The Wolansky et al. (2009) study tests the hypothesis that mixtures of pyrethroids would exhibit dose additivity, when administered at doses below the threshold for induction of the effects as measured in Wolansky et al. (2006). The authors concluded that their results did support dose additivity, using a set of 11 chemicals, five Type I, five Type II and one mixed I/II. These were the same eleven chemicals that the same authors employed in an earlier study (Wolansky et al., 2006) designed to establish their relative potency for acute effects on motor function. The same testing procedure for motor activity was employed in both studies.

Additional research employing motor activity as the endpoint should be considered. Some of the pyrethroids currently registered in the U.S. have not been evaluated for toxicological interactions when applied in mixtures using the study design described in Wolansky et al. (2009). If a preliminary analysis of exposure potential for the pyrethroid group suggests that any one of the untested pyrethroids would contribute significantly (e.g. $\geq$5% of the total) to the overall exposure, then they also should be tested in a mixture interaction study. One way of approaching this would be to apply the test compound in a mixture with two or three of Type I pyrethroids, if the test substance can be classified as Type I, or with two or three Type II pyrethroids, if the test substance can be classified as a Type II, or in a mixture of two or three each of the Type I and Type II pyrethroids tested in the Wolansky et al. (2009) study.

FOB: Existing data suggest that the guideline study design can be tailored to evaluate effects specific to pyrethroids, (see e.g., Nemec 2005, 2006 for some details). There would be value in determining the nature of the interaction for one or more FOB endpoints, alone or in combination, for *all* pyrethroids. The results from these studies could then be compared with those from the EPA studies addressing only motor activity or acoustic startle in order to determine whether the two sets of data are consistent.

Acoustic Startle: Existing data suggest that Type I and II pyrethroids can be distinguished generally on the basis of acoustic startle response: Type I compounds produce increases in startle amplitude; Type II compounds produce decreases (Crofton and Reiter, 1984; Crofton and Reiter, 1988). There would be value in determining whether these distinct patterns of effects are observed across the compounds tested in the "enhanced FOB" study. There may be value in testing the default dose-additivity hypothesis, using a uniform study design, for *all* pyrethroids, to determine if the acoustic startle results offer a more robust distinction between the Type I and Type II pyrethroids, and hence, could be used in additivity studies within each sub-group. The directional nature of the changes, increased acoustic startle reactivity with Type I pyrethroids and decreased reactivity with Type II pyrethroids could confound any interpretation of data based on measures of additivity between compounds from the two sub-groups. It would be necessary to include the expected contribution to total exposure as a factor in experiments where animals are exposed to combinations of Type I and Type II compounds.

Effects on ion channels: The Panel noted that there would be value in performing interaction studies with one or more of the ion channel systems addressed in the EPA Draft Science Policy Paper to test the additivity hypothesis. The EPA Draft Science Policy Paper mentions the ongoing EPA study using microelectrode arrays. Unfortunately, the statistical analysis of the results has not been completed, so the findings could not be shared with the Panel at this time. However, EPA noted that the specific neuronal cell type used in the experimental system possesses all three of the ion channel systems, although only sodium ion channel effects were being measured in the study. Furthermore, the responses to both Type I and Type II pyrethroids were similar to those observed in the motor activity studies, that is, there was a uniform *decrease* in neuronal activity (e.g., spike rate shows few differences between the two groups of compounds). The MEA approach is data rich; EPA suggested that preliminary examination of the data indicated some subtle distinctions between some of the effects of Type I and Type II compounds in this system.

Continued Panel discussion focused on the possibility that the results of the MEA studies might not be fully concordant with the results of the motor activity study. Possible explanations for differences were discussed. The kinetics would be expected to be different in several respects. The compound has direct access to the target site in the MEA system, but not *in vivo*. Different times to peak effect are less of an issue in the *in vivo* environment. The *in vitro* system is a focused culture of a specific type of neuron cells, whereas the whole animal system involved in motor activity is more complex and integrates the activities of large numbers of nerve cells with different properties that interact within a regulated environment. The *in vitro* system may lack critical biological components. Efforts are currently underway to determine the level of concordance between the results of the two systems. The PBPK models under development by the Agency will be critical in the interpretation of such comparisons. However, the outcome of these analyses may indicate a need to obtain additional pharmacodynamic information.

In summary, the Panel recommended that at least one additional mixture study be conducted *in vivo*. Acoustic startle was seen as a good endpoint for evaluation. There was consensus that it would be more appropriate to test chemicals in pairs or small sets, rather than as the 11-component mixture used in the Wolansky et al. (2009) and the ongoing EPA microelectrode array studies. If the Agency were to decide to subgroup the pyrethroids, the study design might consist of two groups of mixtures: one containing Type I only and another containing Type II only. In such a protocol, the Agency should consider measuring a type-specific endpoint, rather than one that is common across types. Alternatively, a thorough evaluation of the effects of each compound on several different endpoints could be considered. The choice of which substances to test in combination should be informed by knowledge of their potential for co-exposure in real life environmental circumstances.

REFERENCES

Barger S.W., M.E. Goodwin, M.M. Porter and M.L. Beggs. 2007. Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *J. Neurochem.* 101: 1205-1213.

Block M.L. and J.-S. Hong. 2005. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Progr. Neurobiol.* 76: 77-98.

Burr, S. E. and D.E. Ray. 2004. Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. *Toxicol. Sci.* 77: 341-346.

Crofton, K.M. and L.W. Reiter. 1984. Effects of 2 pyrethroid insecticides on motor-activity and the acoustic startle response in the rat. *Toxicol. and Appl. Pharm.* 75: 318-328.

Crofton, K.M. and L.W. Reiter. 1988. The effects of Type-I and Type-II pyrethroids on motor-activity and the acoustic startle response in the rat. *Fund. and Appl. Toxicol.* 10: 624-634.

Davalos D., J. Gruntzendler, G. Yang, J.V. Kim, S. Jung, D.R. Littman, M.L. Dustin and W.B. Gan. 2005. ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* 8: 752-758.

Davies, T.G.E., L.M. Field, P.N. Usherwood and M.S. Williamson. 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life*. 59: 151-162.

Garden, G.A. and T. Möller. 2006. Microglia biology in health and disease. *J. Neuroimmune Pharmacol.* 1: 127-137.

Goldin, A. L. 2001. Resurgence of sodium channel research. *Ann. Rev. Physiol.* 63: 871-894

Isom, L.L., 2001. Sodium channel beta subunits: anything but auxiliary. *Neuroscientist.* 7: 42-54.

López-Redondo, F., K. Nakajima, S. Honda and S. Kohsaka. 2000. Glutamate transporter GLT-1 is highly expressed in activated microglia following facial nerve axotomy. *Brain Res. Mol Brain Res.* 76: 429-435.

Lund, A.E. and T. Narahashi. 1981. Interaction of DDT with sodium channels in squid giant axon membranes. *Neuroscience*. 6: 2253-2258.

McDaniel, K.L. and V.C. Moser. 1993. Utility of a neurobehavioral screening battery for differentiating the effects of two pyrethroids, permethrin and cypermethrin. *Neurotoxicol. and Teratol.* 15: 71-83.

Narahashi, T. and H.G. Haas. 1968. Interaction of DDT with the components of lobster nerve membrane conductance. *J. Gen. Physiol.* 51: 177-198.

Nemec, N.D. 2005. A Range-finding Acute Functional Observation Battery Comparison Study in Rats. Pyrethroid Working Group Study. MRID 47050504.

Nemec, N.D. 2006. An acute functional observational battery comparison study in rats. Pyrethroid Working Group Study. MRID 47050505.

Ogata, N., S.M. Vogel and T. Narashasi. 1988. Lindane, but not deltamethrin, blocks a component of GABA-activated chloride channels. FASEB J. 2895-2900.

O'Reilly, A.O., B.P. Khambay, M.S. Williamson, L.M. Field, B.A. Wallace and T.G. Davies. 2006. Modelling insecticide-binding sites in the voltage-gated sodium channel. *Biochem. J.* 396: 255-263.

Peng F., I.R. Mellor, M.S. Williamson, E.T.G. Davies, L.M. Field, and P.N.R. Usherwood. 2009. Single channel study of deltamethrin interactions with wild-type and mutated rat NaV1.2 sodium channels expressed in *Xenopus* oocytes. *Neurotoxicology* 30: 358-367.

Plummer, N. W. and M. H. Meisler. 1999. Evolution and diversity of mammalian sodium genes. *Genomics* 57: 323-31.

Song, J.-H., K. Nagata, H. Tatebayashi, and T. Narahashi. 1996. Interactions of tetramethrin, fenvalerate and DDT at the sodium channel in rat dorsal root ganglion neurons. *Brain Res.* 708: 29-37.

Symington, S.B., R.K. Frisbie, D.L. Kim and J.M. Clark. 2007a. Action of cismethrin and deltamethrin on functional attributes of isolated presynaptic nerve terminals from rat brain. *Pesti. Biochem. Physiol.*. 87: 172-181.

Symington, S.B., R.K. Frisbie, H-J. Kim and J.M. Clark. 2007b. Mutation of threonine-422 to glutamic acid mimics the 13 phosphorylation state and alters the action of deltamethrin on Cav2.2. *Pesti. Biochem. Physiol.* 88: 312-320.

Taylor, D.L., F. Jones, E.S. Kubota, and J.M. Pocock. 2005. Stimulation of microglia metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor α-induced neurotoxicity in concert with microglial-derived fas ligand . *J. Neurosci.* 25: 2945-2964.

Thun, J., A-K. Persson and K. Fried. 2009. Differential expression of neuronal voltage-gated sodium channel mRNAs during the development of the rat trigeminal ganglion., *Brain Res*. 1269: 11-22.

Usherwood, P.N., H. Vais, B.P.S. Khambay, T.G.E. Davies, and M.S. Williamson. 2005. Sensitivity of the *Drosophila* para sodium channel to DDT is not lowered by the super-kdr mutation M918T on the IIS4-S5 linker that profoundly reduces sensitivity to permethrin and deltamethrin. FEBS Lett. 579: 6317-6325.

Usherwood, P. N., T.G. Davies, I.R. Mellor, A.O.O'Reilly, F. Peng, H. Vais, B.P. Khambay, L.M. Field, and M.S. Williamson,. 2007 Mutations in DIIS5 and the DIIS4-S5 linker of *Drosophila melanogaster* sodium channel define binding domains for pyrethroids and DDT. FEBS Lett. 581: 5485-5492.

Vais H., S. Atkinson, N. Eldursi, A.L. Devonshire, M.S. Williamson, and P.N.R. Usherwood. 2000. A single amino acid change makes a rat neuronal sodium channel highly sensitive to pyrethroid insecticides. FEBS Lett. 470: 135-138.

Watanabe H., H. Abe, S. Takeuchi and R. Tanaka. 2000. Protective effect of microglial conditioning medium on neuronal damage induced by glutamate. *Neurosci. Lett*. 289: 53-56.

Wolansky, M. J., C. Gennings and K. M. Crofton. 2006. Relative potencies for acute effects of pyrethroids on motor function in rats. *Toxicol. Sci.* 89(1): 271-277.

Wolansky, M.J., C. Gennings, M.J. DeVito and K.M. Crofton. 2009. Evidence for dose additive effects of pyrethroids on motor activity in rats. Environmental Health Perspectives. http://www.ehponline.org/docs/2009/0900667/abstract.pdf

Yu, F.H. and W.A. Catterall. 2003. Overview of the voltage-gated sodium channel family. *Genome Biol*. 4:207.