AGENDA
U.S. ENVIRONMENTAL PROTECTION AGENCY (EPA)
FIFRA SCIENTIFIC ADVISORY PANEL (SAP)
OPEN MEETING
MAY 19-21, 2015
FIFRA SAP WEB SITE http://www.epa.gov/scipoly/sap/
DOCKET NUMBER: EPA–HQ–OPP–2015–0130
U.S. ENVIRONMENTAL PROTECTION AGENCY
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Scientific Uncertainties Associated with Research to Evaluate the Potential for Juvenile Sensitivity to Pyrethroids

Please note that all times are approximate (see note at end of Agenda).

Day 1
Tuesday, May 19, 2015

9:00 A.M. Opening of Meeting and Administrative Procedures – Fred Jenkins, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. Introduction and Identification of Panel Members – Stephen Klaine, Ph.D., FIFRA Scientific Advisory Panel Chair

9:10 A.M. Welcome and Opening Remarks – Jack Housenger, Director, Office of Pesticides Programs (OPP), EPA

9:15 A.M. Implementing 21st Century Toxicity Testing at EPA's Office of Pesticide Programs – Anna Lowit, Ph.D., Senior Scientist, Health Effects Division (HED), OPP, EPA

9:35 A.M. Evaluation of Potential Juvenile Sensitivity from Pyrethroid Exposure – Monique Perron Sc.D; Jaime D'Agostino, Ph.D.; William Irwin, Ph.D., DABT; Anna Lowit, Ph.D., HED, OPP, EPA

10:00 A.M. Benefits of Pyrethroids, CAPHRA Overview, Conceptual Framework – Thomas G. Osimitz, Ph.D., DABT, ERT, Science Lead: Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA), Principal Scientist Science Strategies, LLC, Charlottesville, VA

10:20 A.M. Pharmacodynamics Introduction and Background – Larry Sheets, Ph.D., DABT Toxicology Fellow, Human Safety Regulatory Toxicology, Bayer CropScience LP, Research Triangle Park, NC

10:35 A.M. Break

11:00 A.M. *In vivo* Acoustic startle response (ASR)/Detailed Clinical Observations (DCO)/Tissue Levels – Charles V. Vorhees, Ph.D., Professor, University of Cincinnati Department of Pediatrics, Cincinnati, OH

11:25 A.M. Human Isolated Voltage Sensitive Sodium Channel (VSSC) – Steven B. Symington, Ph.D., Associate Professor Department of Biology and Biomedical Science, Salve Regina University Newport, RI

11:40 A.M. Neurolemma – J. Marshall Clark Ph.D., Professor Department of Veterinary and Animal Science Director of the Massachusetts Pesticide Analytical Laboratory, University of Massachusetts, Amherst, MA

12:00 P.M. Lunch

1:00 P.M Pharmacodynamics Conclusion – Thomas G. Osimitz, Ph.D., DABT, ERT, Science Lead – Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA), Principal Scientist, Science Strategies, LLC, Charlottesville, VA

1:15 P.M. Pharmacokinetics: Introduction and Background – Thomas G. Osimitz, Ph.D., DABT, ERT, Science Lead – Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA), Principal Scientist, Science Strategies, LLC, Charlottesville, VA

1:25 P.M. Framework and *In Vitro* to *In Vivo* Extrapolation (IVIVE) Paradigm – Harvey J. Clewell III, Ph.D., DABT, FATS Senior Investigator, The Hamner Institutes for Health Sciences, Research Triangle Park, NC

1:45 P.M. Parameterizing the Model Supporting IVIVE, Metabolic Clearance from *In Vitro* Studies, Other inputs – Brian G. Lake, BSc, Ph.D., DSc, FBTS, LFR Molecular Sciences Leatherhead, Surrey, UK; Miyoung Yoon, Ph.D. Senior Research Investigator, The Hamner Institutes for Health Sciences Research Triangle Park, NC

2:30 P.M. Break

2:45 P.M. Building Information to Validate the Model, Model Structure/Logistics/etc. Key Model Drivers (Km, etc.)., Strengths/Weaknesses/Uncertainties of the Model – Miyoung Yoon, Ph.D. Senior Research Investigator, The Hamner Institutes for Health Sciences Research Triangle Park, NC

3:10 P.M. Model Simulations, Rat: Validation, Human, Juvenile: Adult Comparisons, Reverse Dosimetry (Reality Check for Deltamethrin Model) – Harvey J. Clewell III, Ph.D., DABT, FATS, Senior Investigator, The Hamner Institutes for Health Sciences, Research Triangle Park, NC

3:40 P.M. Pharmacokinetics Conclusion – Thomas G. Osimitz, Ph.D., DABT, ERT, Science Lead – Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA), Principal Scientist Science Strategies, LLC, Charlottesville, VA

3:50 P.M. Read Across Proposals for Other Compounds – Thomas G. Osimitz, Ph.D., DABT, ERT, Science Lead – Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA), Principal Scientist Science Strategies, LLC, Charlottesville, VA

4:00 P.M. CAPHRA Conclusions – Thomas G. Osimitz, Ph.D., DABT, ERT, Science Lead – Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA), Principal Scientist Science Strategies, LLC, Charlottesville, VA

4:30 P.M. Adjourn

Day 2
Wednesday, May 20, 2015

9:00 A.M. Opening of Meeting and Administrative Procedures – Fred Jenkins, PhD, Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. Introduction and Identification of Panel Members – Stephen Klaine, PhD, FIFRA Scientific Advisory Panel Chair

9:10 A.M. Follow-up from the Previous Day Presentations

9:30 A.M. Public Comments

10:45 A.M. Break

11:00 A.M. Charge to the Panel

1. **High-throughput screening studies using human sodium channels expressed in mammalian cells with their regulatory Beta subunits (ChanTest Data):** Human voltage-gated sodium channels (NaVs 1.1, 1.2, 1.3, 1.6) were expressed in human embryonic kidney (HEK) cells and 9 pyrethroids were tested for their effects on channel conductance, as well as the effects of co-expressing the Beta 1 and 2 regulatory subunits.

a. Please comment on the ChanTest studies conducted for nine pyrethroids. Please include in your comments a consideration of their robustness (i.e. reproducibility, controls, statistics, background information, NaV selection, etc). Please comment on the confidence and uncertainties in the ChanTest experiments and related findings.

b. CAPHRA thinks that additional data from this line of evidence are unlikely to contribute useful information to characterize the potential for juvenile sensitivity. Thus, the CAPHRA has proposed NOT to continue this line of research. Please comment on this proposal and degree to which the ChanTest data inform the issue of evaluating the potential for juvenile sensitivity.

c. The ChanTest experiments focus on human sodium channels and generally show weak response. In contrast, *in vitro* studies in rodents in the literature (e.g. Choi and Soderlund, 2006; Meacham et al., 2008; Tan and Soderlund, 2009) show stronger responses to pyrethroids. Please comment on the extent to which the expressed human sodium channels can be used in combination with these sources to infer relative pharmacodynamic sensitivity between rats and humans.

12:00 PM Lunch

1:00 P.M. Charge to the Panel

2. Transplantation of adult & juvenile rat brain synaptic membrane into Xenopus oocytes: Purified neurolemma membranes from adult and juvenile rats were separately micro-injected into Xenopus oocytes. Patch clamp testing was performed on the oocyte membranes versus various doses of pyrethroids to determine their EC50 values for NaV channel activation. Inhibitors of competing channels were added to isolate the sodium channel conductance (i.e. chloride and calcium channels).

a. Please comment on the synaptic membrane ("neurolemma") studies conducted for deltamethrin and permethrin. Please include in your comments a consideration of the robustness of data from the synaptic membrane transplantation into oocytes (reproducibility, control compounds, channel modulator reagents, statistics, use of Na blocker tricaine as an anesthetic, etc) and the degree to which these data inform the issue of evaluating the potential for juvenile sensitivity. Please comment on the confidence and uncertainties and associated findings in the oocyte experiments.

b. In the context of your response to 2a, the CAPHRA has proposed to collect dose-response data in adult and juvenile rat synaptic membrane transplanted into oocytes for five more pyrethroids (including Type I, Type II, and mixed). CAPHRA's proposed path forward: If the additional five pyrethroids show similar patterns to deltamethrin and permethrin (i.e. no lifestage sensitivity observed), no additional pyrethroids will likely be tested in this system. Alternatively, if a different pattern is observed, additional pyrethroids are likely to be tested. Please comment on the CAPHRA's proposed path forward.

2:00 P.M. Charge to the Panel

3. Targeted *in vivo* studies in adult and juvenile rat: acoustic startle/detailed clinical observations: Preliminary experiments that measure acoustic startle and detailed clinical observations have been conducted by the University of Cincinnati. In these experiments,

juvenile and adult male Sprague-Dawley rats were treated with deltamethrin and permethrin as model Type II and Type I pyrethroids, respectively. In addition, brain and plasma concentrations were measured in PND 15 and 90 rats exposed to deltamethrin. The preliminary results suggest that juvenile rats are more sensitive to deltamethrin based on changes in detailed clinical signs and, to less extent, acoustic startle. However, there was no greater sensitivity in juvenile rats exposed to permethrin. Preliminary data from whole brain tissue samples with deltamethrin indicated that PND 15 rats had higher deltamethrin concentrations as compared to adult rats given the same dose. The brain concentration and toxicity data from the CAPHRA studies for deltamethrin are consistent to those previously published by the Bruckner lab (Kim et al., 2010) which showed that at a similar dose (2 mg/kg deltamethrin) PND 10 rats had increased severity of clinical signs and higher Cmax of deltamethrin in the brain as compared to PND 21, PND 40, and PND 90 rats.

a. The *in vivo* behavior studies reported thus far are preliminary evaluations. Please comment on the study design in the preliminary *in vivo* studies for deltamethrin and permethrin conducted at the University of Cincinnati (Vorhees lab).

b. In 2010, FIFRA SAP commented on some challenges that were anticipated with the use of auditory startle to compare the relative sensitivity of juveniles and adults. In the studies conducted by the CAPHRA, both auditory startle and detailed clinical observations were evaluated. Please comment on the degree to which the auditory startle and detailed clinical observations provide useful data for evaluating the potential for juvenile sensitivity. Please include in your comments discussion of the dynamic range; the direction of the response varying between Type I and IIs; and the type of data obtained (continuous, ranked).

2:45 P.M. Break

3:00 P.M. Charge to the Panel

c. Please provide comments comparing the temporal pattern and magnitude of the brain and plasma concentration data from the Vorhees & Bruckner labs and utility of such data to aid in the interpretation of the auditory startle and detailed clinical observation data.

d. Please comment on the use of a 5 ml/kg dosing volume in the CAPHRA studies and how any impact on pyrethroid kinetics affects correlations with behavioral effects.

3:45 P.M. Charge to the Panel

4. Pharmacokinetic studies: A number of pharmacokinetic studies using deltamethrin were performed by the CAPHRA in order to further refine and validate the developing rat PBPK model and construct a developing human PBPK model. For refinement of the rat PBPK model, tissue: plasma partition coefficients (in vivo measurements in PND 21 and adult rats), age dependent plasma protein binding (in vivo measurements in PND 10, 15, 21, and 90 rats), and cytochrome P450 (CYP) and carboxylesterase (CES) metabolism (in vitro measurements using rat liver and plasma preparations from PND 15, 21, and 90 rats) were evaluated. Further in vivo pharmacokinetic studies in rats were conducted with single IV (PND 90), single oral (PND 90),

and multiple oral (PND 15, 21, and 90) doses to generate plasma and tissue data to validate the developing rat PBPK model. Additional experiments with deltamethrin were conducted for constructing the developing human PBPK model. Parameters evaluated in vitro included age dependent plasma protein binding derived in plasma from human donors aged from birth to adults, transport across the blood brain barrier using a human brain microvascular endothelial cell line, estimates of gastrointestinal absorption in caco-2 cells, and ontogeny data for CES CYP1A2, and CYP2C8 determined in human liver tissue from donors (age 1-18 years old). Further *in vitro* experiments are currently underway by the CAPHRA to determine human adult and juvenile liver metabolism of deltamethrin by CYP and CES enzymes using human liver preparations or recombinant human enzymes.

a. Please comment on the *in vitro* experiments to support the PBPK model development in the rat conducted thus far for deltamethrin.

b. Please comment on the *in vivo* experiments to support the PBPK model development in the rat conducted thus far.

c. Please comment on the *in vitro* experiments in the human tissue conducted thus far. Please include in your comments a discussion of the ongoing in vitro experiments with recombinant enzymes for use in PBPK models and associated confidence and uncertainty with the use of such data.

4:30 P.M. Charge to the Panel

5. Physiologically-based pharmacokinetic model in rat: Using data described in Question 4, the CAPHRA has developed a PBPK model for deltamethrin using age-specific metabolism parameters in rats to simulate plasma and brain internal exposures in young and adult rats. This PBPK model relies on *in vitro* to *in vivo* extrapolation (IVIVE) to use age-specific metabolic data collected in vitro to estimate hepatic metabolic clearance in vivo. The deltamethrin brain or plasma concentrations estimated by the model in rats are then compared to measured concentrations from *in vivo* rat studies to verify the model. Non-chemical specific physiological parameters for rats were obtained from the published literature, including body weight, cardiac output, hematocrit levels, tissue volumes, and tissue blood flows. As discussed in Question 4, recently generated data by the CAPHRA and published data were used for compound-dependent parameters, including partition coefficients, metabolic rate constants, absorption rates, protein binding, compartments and tissue permeability.

a. Please comment on the robustness of the rat PBPK model for simulating internal exposures in the developing rat. In your response, please include evaluation of the structure and parameters used to build the model, as well as its ability to accommodate different oral absorption scenarios (i.e. different vehicles used for in vivo studies) and discussion of confidence, accuracy and uncertainties associated with the deltamethrin developing rat model. Please also comment on the sensitivity analyses of parameters CAPHRA has completed thus far.

b. In the context of your response to 5a, please comment on the extent to which additional data are/are not needed to refine the developing rat model.

5:15 PM Adjourn

Day 3
Thursday, May 21, 2015

9:00 A.M. Opening of Meeting and Administrative Procedures – Fred Jenkins, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. Introduction and Identification of Panel Members – Stephen Klaine, Ph.D., FIFRA Scientific Advisory Panel Chair

9:10 A.M. Charge to the Panel

6. Physiologically-based pharmacokinetic model for human: Similar to the rat PBPK model, the human PBPK model integrates non-chemical specific physiological parameters for humans from the literature. Compound-dependent parameters, such as partition coefficients and oral absorption parameters, were adapted from the rat PBPK model. Recently generated data by the CAPHRA were used for the remaining compound-dependent parameters, such as metabolic rate constants, protein binding and tissue permeability. With respect to metabolic constants, CYP and CES enzymes involved in metabolism of a given pyrethroid will be identified and the *in vitro* metabolic constants for those enzymes will be determined for integration into the PBPK model. Intrinsic clearance for each active enzyme will be scaled to *in vivo* using scaling factor data collected by the CAPHRA and the SIMCYP database. The ontogeny of enzyme expression (also from the CAPHRA data and the SIMCYP database) will be incorporated into the process of obtaining distributions of age-specific intrinsic clearance for each enzyme. Preliminary simulations have been conducted for deltamethrin to demonstrate the process used with the PBPK model.

a. Within the context of understanding potential juvenile sensitivity, characterize the robustness of the PBPK model for extrapolating age-specific internal tissue exposures for humans. In your response, please comment on the structure and parameters used to build the model and include discussion of confidence, reliability, and uncertainties associated with the deltamethrin human model. Please include in your comments a discussion of the data from the McCarver/Hines Laboratory (submitted as part of the CAPHRA package) for providing ontogeny of CES enzymes.

b. Please comment on the proposed use of SIMCYP for providing enzyme ontogeny patterns and deriving population distributions for metabolic parameters in humans. Please include in your comments whether or not other tools with similar capacity to SIMCYP are available.

10:00 A.M. Charge to Panel

7. Physiologically-based pharmacokinetic models for humans with other pyrethroids: Thus far, the CAPHRA has focused its human PBPK efforts on deltamethrin and, to a lesser extent, permethrin. Soon laboratory efforts will turn to other pyrethroids. CAPHRA's proposal is to conduct fewer studies and in lieu of such data, use read across and computational approaches.

a. Please comment on the appropriateness of the current human PBPK model to be used for other Type I, Type II, or mixed type pyrethroids. Please include in your response evaluation of the path forward provided by the CAPHRA regarding *in vitro* and *in vivo* studies for other Type I, Type II, or mixed type pyrethroids.

10:30 A.M. Break

10:45 A.M. Charge to Panel

8. Integration of lines of evidence:

a. The tissue dosimetry data from the rat suggest higher brain levels in juveniles compared to adults. In contrast, the preliminary PBPK modelling for human predicts slightly lower brain concentrations in young children compared to adults. Please comment on these differences, including comments on the key inputs that lead to this difference, human variability associated with the key parameters, and the confidence and uncertainties associated with the difference between the rat and human internal dosimetry.

b. The CAPHRA has proposed to continue *in vivo* behavioral testing for deltamethrin and permethrin in definitive dose-response evaluations. Pyrethroids have been studied for decades and thus there is a large body of evidence for these pesticides. Given your response to 8a along with 1) the extensive body of scientific literature on pyrethroid toxicity syndromes and high dose studies in juvenile rats (e.g., Sheets et al, 1994); 2) neurolemma studies supported by the CAPHRA (Question 2) along with additional *in vitro* studies (e.g. Meacham et al., 2008); and 3) recent *in vivo* studies from the Vorhees & Bruckner labs, please comment on the additional scientific value that would be provided in conducting further *in vivo* rat experiments to assess potential for PD sensitivity of human infants and children.

12:00 PM Lunch

1:00 P.M. Charge to Panel

c. If you believe there is additional scientific value to conducting additional *in vivo* experiments (8b), please comment on the CAPHRA's proposed experiments. In the context of your response to Question 3, please include in your comments a discussion of dose levels and/or additional study design elements to improve existing preliminary evaluations.

1:30 P.M. Closing Remarks – Stephen Klaine, PhD, FIFRA Scientific Advisory Panel Chair; FIFRA SAP members; US EPA; Fred Jenkins, PhD, Designated Federal Official, Office of Science Coordination and Policy, EPA

2:00 P.M. Meeting Adjourn

Please be advised that agenda times are approximate; when the discussion for one topic is completed, discussions for the next topic will begin. For further information, please contact the Designated Federal Official for this meeting, Dr. Fred Jenkins, via telephone: (202) 564-3327; fax: (202) 564-8382; or email: jenkins.fred@epa.gov.