AGENDA
FIFRA SCIENTIFIC ADVISORY PANEL (SAP)
OPEN MEETING

July 23, 2010

FIFRA SAP WEB SITE http://www.epa.gov/scipoly/sap/
OPP Docket Telephone: (703) 305-5805
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U.S. Environmental Protection Agency
Conference Center - Lobby Level
One Potomac Yard (South Bldg.)
2777 S. Crystal Drive, Arlington, VA 22202

Scientific Issues related to the Comparative Adult and Juvenile Sensitivity Toxicity Protocols for Pyrethroids

Please note that all times are approximate
(See note at the end of the Agenda)

Friday, July 23, 2010

8:30 A.M. **Opening of Meeting and Administrative Procedures by Designated Federal Official –** Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

8:35 A.M. **Introduction and Identification of Panel Members –** Steven Heeringa, Ph.D., Chair, FIFRA Scientific Advisory Panel

8:40 A.M. **Welcome and Opening Remarks –** Tina Levine, Ph.D., Director, Health Effects Division, Office of Pesticide Programs, EPA

9:00 A.M. **Regulatory Overview and Objectives –** Edward Scollon, Ph.D., Health Effect Division, Office of Pesticide Programs, EPA

10:00 A.M. **BREAK**

10:15 A.M. **PUBLIC COMMENTS**

11:15 A.M. **Charge Question 1**

1.0 Auditory Startle Response or Acoustic Startle Reflex (ASR)

1.1 The auditory startle is a commonly used technique to assess neurobehavioral effects in rats. Auditory startle reflex is a motor reflex characterized by a sequence of reflexive muscle movements elicited by sudden and intense acoustic stimuli measured by a change in motor output. The proposed reflex path is short, consisting of the

auditory nerve, posteroventral cochlear nucleus, the nucleus reticularis pontis caudalis, and motor neurons in the spinal cord (Davis et al. 1982). This mechanism is susceptible to a variety of drugs and toxicants making the reflex a useful model of sensorimotor reactivity across animal taxa, including rat and human (Lee et al. 1996). With regard to pyrethroids, auditory startle data in adult rats have demonstrated differing response patterns related to pyrethroid structure (Crofton and Reiter 1984; Tilson et al. 1985; Crofton and Reiter 1988; Hijzen et al. 1988; Hijzen and Slangen 1988); Type I pyrethroids produced an increase in startle amplitude and Type II pyrethroids produced a decrease in startle amplitude. In addition, ASR has been used to demonstrate age-dependent toxicity in rats following high oral doses of pyrethroids (Sheets et al. 1994; Sheets 2000). Therefore, ASR is a potentially sensitive measure to evaluate differences in neurobehavioral effects between adults and pups.

Since ASR is a behavioral measurement, it is important to consider development, dose-response and variability during interpretation of the results. In rats, the onset of ASR response corresponds to the development of the external auditory meatus. In the rat, this usually occurs between 13 and 16 days of age. Sheets et al. (1988) have shown the ability of rats to respond to ASR as early as PND 13, however, the amplitude of response continued to increase through PND 21. Pyrethroids modify the voltage gated sodium channels in the central nervous system and therefore the brain is considered the major target organ for toxicity. Kim et al. (2010) determined the distribution of deltamethrin, a Type II pyrethroid, in brain, fat, liver, plasma, and muscle in PND 10, 21, 40, and 90 rats for up to 510 hours. Brain concentrations in PND 10 pups were elevated for a longer time relative to the adults. This suggests that pyrethroid kinetics in the brain of pups may not mirror those of adult rats. The Kim et al. (2010) study emphasizes the importance of determining the appropriate time course of effects (i.e., time-to-peak-effect and/or time-to-tissue-recovery) in both adult and non-adult lifestages prior to measuring ASR responses. Additionally, the standard deviation for peak amplitude, the ASR measure proposed by the PPTWG, can vary greatly in guideline DNT studies (20-125%) and literature reviews (Raffaele et al. 2004). However, this variability can be reduced down to 20-30% if the studies are conducted in proven laboratories (Sette et al. 2004).

The PPTWG is proposing that the ASR provides a robust and sensitive measure of neurotoxicity and is well suited to assess age-dependent sensitivity to pyrethroids. Please comment on the appropriateness of the ASR technique as a measure of pyrethroid induced toxicity, including suggestions to assure quality of the study design (i.e., appropriate time-to-peak response, variability of peak response, etc.) and resulting data.

1.2 Age-dependent toxicity has been observed in rat studies following high doses (i.e., LD_{50} studies resulting in 50% mortality of test subjects) of Type II pyrethroids (Cantalamessa 1993; Sheets et al. 1994; Sheets 2000). However, in sublethal studies using the ASR as a measure of toxicity, ED_{50} (dose at which 50% of the test subjects are affected) values were similar between postnatal day (PND) 21 and adult rats.

These findings suggest that age-dependent toxicity may only be observed at high doses. Based on *in vivo* (Cantalamessa 1993) and *in vitro* (Anand et al. 2006) studies, the apparent discrepancy between high- and low-dose age-dependent toxicity is likely attributable to incomplete maturation of the enzymes that detoxify pyrethroids in immature animals, particularly the carboxylesterases and cytochrome P450s. These clearance mechanisms are overwhelmed in younger animals given LD50 doses, leading to increased accumulation of the pyrethroids in nervous tissue and ultimately increased toxicity.

Carboxylesterases and P450 enzymes are the two major enzyme families responsible for metabolism of pyrethroids. In the rat, it has been shown that carboxylesterase activities are below adult levels at weaning (Moser et al. 1998; Karanth and Pope 2000; Anand et al. 2006; de Zwart et al. 2008; Yang et al. 2009). Information on the ontogeny of carboxylesterase development in the human is more limited. However, increased plasma esterase activity during postnatal maturation has been reported (Ecobichon and Stephens 1973). In contrast, Pope et al. (2005) found carboxylesterase activity in hepatic tissues were similar for humans ranging in age from 2 months to 36 years, however, the sample sizes were small and variability among the age groups was high. Maturation of the P450 enzymes shows a similar trend. 2C19, a P450 enzyme which has shown high pyrethroid metabolic activity (Godin et al. 2006), increases rapidly in the human during first 2 years of life, whereas numerous P450s examined in the rat have minimal expression levels through gestation and do not approach adult levels of expression until PND10 days or later (de Zwart et al. 2008).

Comparisons between lifestages in the rat and human are difficult because of the ontogeny of the brain development and metabolizing enzymes are not an exact match. However, PND 11 rats are considered to be close in development to newborn humans and PND 17 rats are believed to be closer developmentally to human toddlers (Davision and Dobbing 1966; Dobbing and Smart 1974; Benjamins and McKhann 1981). From the aspect of exposure, previous experience with developing cumulative risk assessments for other insecticide groups, ongoing work on HED's *Standard Operating Procedures for Residential Pesticide Exposure Assessment*, and the Agency's *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures for Environmental Contaminants* http://www.epa.gov/raf/publications/guidance-on-selecting-age-groups.htm, the Agency believes that children three years old and younger, particularly those who are mobile (crawling, walking) and who exhibit hand-to-mouth behavior, have the potential for the greatest exposure to pyrethroids. Based on 1) the current understanding that the two major enzyme families responsible for the metabolism of pyrethroids are below adult activity levels at weaning (i.e., PND 21); 2) PND 17 rats are approximately comparable to human toddlers in terms of development; and 3) children younger than 3 years of age are expected to have the greatest exposures to pyrethroids, the Agency is concerned with the PPTWG's proposal to conduct ASR studies in PND 21 rats. Instead, PND 15 to 17 rats, which have been shown to respond to ASR stimuli (Sheets et al. 1988), may better represent the most susceptible human lifestage.

The PPTWG has proposed to use conduct ASR studies on 21-day old rats. **Please comment on the appropriateness of this age group in regards to *i*) assessing age-dependent toxicity and *ii*) assessing whether the 21-day old rat will adequately inform the Agency regarding toxicity as it relates to children three years of age and younger.**

12:00 P.M. LUNCH

1:00 P.M. Charge Question 1 cont'd

2:00 P.M. Charge Question 2

2.0 Physiologically-Based Pharmacokinetic (PBPK) Modeling

The PPTWG is proposing to use a model developed collaboratively by EPA's Office of Research and Development (ORD) and the University of Georgia as a starting point in their modeling effort. EPA's ORD has published a series of papers that describe the development and enhancement of pyrethroid PBPK models starting with a deltamethrin model in rats by Mirfazaelian et al. (2006) , improved by Godin et al.(2010), modified for permethrin by Tornero-Velez et al. (*in prep.*), and finally expanded to include age- and chemical-dependent parameters by Tornero-Velez et al. (2010). In 2007, ORD and OPP jointly presented an issue paper to the SAP (USEPA 2007) which described an approach for using a generic model structure with chemical specific parameters for pyrethroids. The "family modeling" approach was endorsed by the SAP and has been successfully applied in the above PBPK efforts. The Agency believes that it is both reasonable and scientifically sound to use the Tornero-Velez et al. (2010) PBPK model as the starting point for the PPTWG effort to build PBPK models for pyrethroids to assess young children. Furthermore, the PPTWG is proposing to develop PBPK models using *in vitro* and *in vivo* rat data, and then using human *in vitro* data to inform model to predict human internal dosimetry, similar to the approach which was previously supported by the 2007 SAP.

2.1 The PPTWG proposes to increase the complexity of the Tornero-Velez et al. (2010) PBPK model by modifying some aspects. For example, the PPTWG is proposing to:

a. Predict intestinal permeability through the use of Ussing Chamber technique with rat cells and human Caco-2 cells, with the potential to increase the number of compartments within the intestinal tract
b. *In vitro* determination of partition coefficients
c. Obtain estimates of protein binding

Please comment on the proposed modifications to the Tornero-Velez et al. (2010) model as described in sections 4.3 of the PPTWG proposal. Please include in

your comments consideration for balancing potentially improved performance resulting from the increased complexity with model parsimony.

2.2 Microsomal incubation studies have been used to inform the pyrethroid PBPK models developed by ORD (Mirfazaelian et al. 2006; Scollon et al. 2009; Godin et al. 2010; Tornero-Velez et al. 2010; Tornero-Velez *in prep.*). The PPTWG has proposed to use intact hepatocytes instead because they may provide a better prediction of metabolism compared to microcellular fractions (Hewitt et al. 2007). Additionally, the PPTWG is proposing to compare the clearance activity of human hepatocytes and microsomal fractions for several age groups. However, the Agency notes that there are a limited number of human hepatocyte samples available to inform the PBPK model. Pooled human microsomes are available representing larger segments of the population relative to hepatocyte availability. The Working Group suggests characterization of the hepatocytes (i.e., cypP450 and esterase composition) and establishing a relationship between hepatocytes and microsomal fraction activities may reduce model uncertainty in light of limited data.

Please comment on the strengths and weaknesses of the PPTWG proposal to use hepatocytes in the PBPK effort, including the potential for hepatocytes to decrease uncertainty of model predictions in light of limited data.

3:15 P.M. BREAK

3:30 P.M. Charge Question 3

3.0 Alternative Study Design(s) For Evaluating Age Differences in Pharmacokinetics

The Agency gives special consideration to the potential pre- and postnatal lifestages regarding potential exposure to pesticides. Pre-natal exposure to pyrethroids has been evaluated extensively in over 80 developmental toxicity, reproductive toxicity, and DNT test guideline studies and no sensitivity from *in utero* exposure has been observed. As previously described, there are gaps in knowledge surrounding the potential for post-natal sensitivity and, as described in Question 1.2, the Agency considers children less than 3 years of age to be the most susceptible population. The PPTWG has proposed a robust PBPK model development effort to describe pyrethroid dosimetry across several lifestages; however, these models will not be ready for use by the Agency until approximately 2013.

Are there alternative approaches using empirical or data generation techniques potentially requiring less time than the PBPK effort proposed by the PPTWG for evaluating the potential for post-natal sensitivity, particularly with respect to differences in pharmacokinetic profiles, that could be used by the Agency?

5:00 P.M. 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. Sharlene Matten, via telephone: (202)-564-0130; fax: (202) 564-8382; or email: matten.sharlene@epa.gov.