July 19, 2002

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held June 26-27, 2002

TO: Marcia E. Mulkey, Director
Office of Pesticide Programs

FROM: Paul I. Lewis, Designated Federal Official
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Larry C. Dorsey, Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Sherell A. Sterling, Acting Director
Office of Science Coordination and Policy

Please find attached the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia from June 26-27, 2002. This report addresses a set of scientific issues being considered by the Environmental Protection Agency regarding determination of the appropriate FQPA Safety Factor(s) in the organophosphorous pesticide cumulative risk assessment: susceptibility and sensitivity to the common mechanism, acetylcholinesterase inhibition.

Attachment

cc:

Stephen Johnson
Susan Hazen
Adam Sharp
James Jones
Janet Andersen
Debbie Edwards
Anne Lindsay
Steve Bradbury
Denise Keehner
Linda Moos
Lois Rossi
Frank Sanders
Margaret Stasikowski
William Jordan
Antonio Bravo
Douglas Parsons
David Deegan
Vanessa Vu (SAB)
OPP Docket

FIFRA Scientific Advisory Panel Members
Stephen M. Roberts, Ph.D.
Fumio Matsumura, Ph.D.
Herbert Needleman, M.D.
Christopher J. Portier, Ph.D.
Mary Anna Thrall, D.V.M.

FQPA Science Review Board Members

John Bigbee, Ph.D.
William Brimijoin, Ph.D.
Amira T. Eldefrawi, Ph.D.
Jean Harry, Ph.D.
Dale Hattis, Ph.D.
George Lambert, M.D.
Michael McClain, Ph.D.
Carey Pope, Ph.D.
Nu-May Ruby Reed, Ph.D.
Lester Sultatos, Ph.D.

SAP Meeting Minutes No. 2002-03

June 26-27, 2002
FIFRA Scientific Advisory Panel Meeting, held at the Sheraton Crystal City Hotel, Arlington, Virginia

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

Determination of the Appropriate FQPA Safety Factor(s) in the Organophosphorous Pesticide Cumulative Risk Assessment: Susceptibility and Sensitivity to the Common Mechanism, Acetylcholinesterase Inhibition

NOTICE

This report has been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). This report has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of this report 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 was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad-hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Larry Dorsey, SAP Executive Secretary, via e-mail at dorsey.larry@.epa.gov.

SAP Meeting Minutes No. 2002-03

FIFRA Scientific Advisory Panel Meeting, June 26-27, 2002, held at the Sheraton Crystal City Hotel, Arlington, Virginia

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

Determination of the Appropriate FQPA Safety Factor(s) in the Organophosphorous Pesticide Cumulative Risk Assessment: Susceptibility and Sensitivity to the Common Mechanism, Acetylcholinesterase Inhibition

Mr. Paul Lewis
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: July 19, 2002

Stephen M. Roberts, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date: July 19, 2002

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
June 26-27, 2002

Determination of the Appropriate FQPA Safety Factor(s) in the Organophosphorous Pesticide Cumulative Risk Assessment: Susceptibility and Sensitivity to the Common Mechanism, Acetylcholinesterase Inhibition

PARTICIPANTS

FIFRA SAP Session Chair
Stephen M. Roberts, Ph.D., University of Florida, Gainesville, FL

Designated Federal Official
Mr. Paul Lewis, FIFRA Scientific Advisory Panel Staff, Office of Science Coordination and Policy

FIFRA Scientific Advisory Panel
Fumio Matsumura, Ph.D., University of California at Davis, Davis, CA
Herbert Needleman, M.D., University of Pittsburgh, Pittsburgh, PA
Christopher J. Portier, Ph.D., National Institute of Environmental Health Science
Research Triangle Park, NC
Mary Anna Thrall, D.V.M., Colorado State University, Fort Collins, CO

FQPA Science Review Board Members

John Bigbee, Ph.D., Virginia Commonwealth University, Richmond, VA
William Brimijoin, Ph.D., Mayo Clinic and Medical School, Rochester, MN
Amira T. Eldefrawi, Ph.D., University of Maryland School of Medicine, Baltimore, MD
Jean Harry, Ph.D., National Institute of Environmental Health Science, Research Triangle Park, NC
Dale Hattis, Ph.D., Clark University, Worcester, MA
George Lambert, M.D., Environmental and Occupational Health Sciences Institute, UMDNJ, Piscataway, NJ
Michael McClain, Ph.D., McClain and Associates, Randolph, NJ
Carey Pope, Ph.D., Oklahoma State University, Stillwater, OK
Nu-May Ruby Reed, Ph.D., California Environmental Protection Agency, Sacramento, CA
Lester Sultatos, Ph.D., New Jersey Medical School, Newark, NJ

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to determination of the appropriate FQPA Safety Factor(s) in the organophosphorous pesticide cumulative risk assessment: susceptibility and sensitivity to the common mechanism, acetylcholinesterase inhibition.

Advance notice of the meeting was published in the *Federal Register* on May 31, 2002. The review was conducted in an open Panel meeting held in Arlington, Virginia, on June 26-27, 2002. The meeting was chaired by Dr. Stephen Roberts. Mr. Paul Lewis served as the Designated Federal Official.

Before the Agency presentation on issues pertaining to determination of the appropriate FQPA safety

factor, Mr. Francis B. Suhre (Office of Pesticide Programs, EPA) provided the Panel a status report on organophosporus pesticide cumulative risk estimates: comparison of outputs from different models.

Vicki Dellarco, Ph.D. (Office of Pesticide Programs, EPA), began the Agency presentations by providing an introduction and overview of the approach to evaluating susceptibility/sensitivity of children in cumulative risk assessments and review of available animal studies. Stephanie Padilla, Ph.D. (Office of Research and Development, EPA) summarized age dependent sensitivity and susceptibility. Vicki Dellarco, Ph.D. (Office of Pesticide Programs, EPA) ended the Agency presentation by discussing the risk characterization of sensitivity and susceptibility. Other EPA participants were Randy Perfetti, Ph.D. (Office of Pesticide Programs, EPA) and Karl Baetcke, Ph.D. (Office of Pesticide Programs, EPA).

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

PUBLIC COMMENTERS

Oral statements were made by:

Jennifer Sass, Ph.D., on behalf of the Natural Resources Defense Council

Angelina Duggan, Ph.D., on behalf of Sound Science Policy Alliance

Larry Sheets, Ph.D., Bayer Crop Science, on behalf of CropLife America

James Gibson, Ph.D., The Brody School of Medicine of Eastern Carolina University, on behalf of Sound Science Policy Alliance

Jack M. Zabik, Ph.D., Dow AgroSciences, on behalf on CropLife America

Mr. Ed Gray, McDermott Will and Emery, on behalf of FQPA-Implementation Working Group

Mr. Art Beltrone, private citizen

Judith Schreiber, Ph.D. State of New York, Office of the Attorney General

Written statements were received as follows:

No written comments were received.

CHARGE

Issue 1. Role of Cholinesterases and Acetylcholine

As discussed in the EPA report, inhibition of acetylcholinesterase (AChE) in the young can result in cholinergic toxicity as in the adult, but evidence has also been emerging over the last several years that indicates that AChE and acetylcholine may serve as neuromodulators in development.

Question 1.1

Please comment on the extent to which the report adequately summarizes the current state of knowledge. Does the scientific evidence support the conclusion that perturbation of the cholinergic nervous system during development by inhibiting AChE can potentially lead to deficits in the structure and function of the central and peripheral nervous systems?

Issue 2. Age-Dependent Sensitivity to Cholinesterase inhibition in Animal Studies

Age-dependent sensitivity (*i.e.*, young animals can exhibit higher levels of cholinesterase (ChE) inhibition at the same dose or inhibition at lower doses compared to adults) has been observed in several laboratory studies following treatment (acute and/or repeated oral gavage doses) of neonatal, juvenile, and adult rats with organophosphorus (OP) pesticides. The exact mechanisms of this age-dependent sensitivity are not known, but several studies have demonstrated that toxicokinetic factors may be responsible. Most notably, the more limited ability of the young to detoxify OP pesticides by A-esterases and carboxylesterases appears to be an important factor underlying the increased sensitivity of the immature rat to ChE inhibition. There appears to be more rapid recovery of inhibited AChE (synthesis of new ChE enzyme) in postnatal (and fetal) rat tissues, but information on comparative recovery in children and human adults is lacking.

Question 2.1

Please comment on the extent to which the report adequately discussed and summarized the current understanding of age-dependent sensitivity to ChE inhibition, the prevailing views in the scientific community concerning the biological factors involved, and the role esterases may play as a major factor accounting for potential increased sensitivity of the immature rat.

Question 2.2

Please comment on the timing of administration (i.e., the developmental stage treated) and the differential found between adults and the young animal.

Question 2.3

Please comment on the extent to which comparative ChE data on six OP pesticides (chlorpyrifos, diazinon, dimethoate, methamidophos, malathion, methyl parathion) may represent a reasonable subset of different structural and pharmacokinetic characteristics of the cumulative group of OP pesticides to define an upper bound on the differential sensitivity that may be expected at different life stages of the immature animal. As an example, there are no chemical-specific comparative cholinesterase data on azinphos-methyl (AZM), an important contributor of risk for the food pathway. Pesticide-specific comparative cholinesterase data on the other six pesticides from the OP class (including data on malathion, a member of the same chemical subgroup as AZM) show a limited range of differential sensitivities -- from one-fold (no increased sensitivity) up to three-fold -- between the young and adults. EPA regards these data on other OPs as providing sufficient evidence to assess the potential for AZM to show age-dependent sensitivity, and to reasonably predict the degree of potential difference in sensitivity between the young and adults. Given the results of the other OPs, EPA concludes that it is unlikely that AZM would exceed a magnitude of difference greater than approximately 3-fold following treatment of PND 11 through 21 pups versus adult animals.

Issue 3. Relevance of the Animal Findings to Children

Age dependent sensitivity to cholinesterase inhibition has been associated with the limited ability of the immature rat to detoxify OP pesticides by esterases. In rats, A-esterase activity increases from birth to reach adult levels around postnatal day 21. Fetal rats possess very little carboxylesterase activity with increasing activity as the postnatal rat matures, reaching adult values after puberty (50 days of age). Data showing increased sensitivity of the young animal to cholinesterase inhibition compared to adults has generally been derived from acute dosing of PND 7 or PND 11 pups, or repeated dosing of PND 11 to PND 21 pups. The available data also show as the young rat rapidly matures in its ability to detoxify by esterases, the differential in cholinesterase inhibition becomes smaller. Thus, the relative sensitivities of immature rats found in the studies of dosing pups through PND 11 to 21 are smaller compared to studies of dosing a PND 11 pup. The dosing studies of PND 11 through 21 pups are considered to better approximate the maturation profile of the A-esterases of the highly exposed children's age group in the OP cumulative risk assessment, the one and two year olds, compared to a study of a PND 11 pup which is similar to a newborn. Thus, the repeated rat dosing studies more closely mimic the maturation or developmental profile of A-esterase appearance in children around the one and two year olds where children are reaching adult levels of A-esterase activity. The use of dosing studies of PND 11 through 21 is consistent with the exposure patterns of children. Humans generally do not begin to consume fresh (uncooked) fruits and vegetables until after six months of age or more. Furthermore, repeated dosing studies were used to determine relative sensitivity because people are exposed every day to an OP pesticide through food, and thus an animal study using repeat exposures is considered appropriate. Finally, following exposure to an OP, regeneration of cholinesterase to pre-exposure levels does not occur for days or weeks, making the exposed individual potentially more vulnerable to subsequent exposures during that period.

Question 3.1

Please comment on the maturation profile of A-esterase and the uncertainties surrounding these data in young children. Because no human data are available on the maturation profile of carboxylesterases, please comment on what should be assumed in humans, especially children age 1 to 2 years, given the animal data and what science understands in general about detoxification maturation profiles.

Question 3.2

Please comment on the extent to which the biological understanding of observed age-dependent sensitivity to cholinesterase inhibition in laboratory animal studies informs our understanding about the likelihood of similar effects occurring in children; in particular, what can be inferred from animal and human information regarding the potential for different age groups to show increased sensitivity if exposed to cholinesterase-inhibiting pesticides. Does the scientific evidence support the conclusion that infants and children are potentially more sensitive to organophosphorus cholinesterase inhibitors?

Question 3.3

Please comment on the conclusions regarding the faster recovery in the young animal of AChE activity. Because there is no human information on the recovery of AChE in children compared to adults, please comment on the extent to which recovery of AChE in children should be factored into conclusions regarding potential risk to children.

PANEL CONSIDERATION OF AGENCY
APPLICATION OF THE FQPA SAFETY FACTOR

The Panel was not explicitly charged with making a determination as to whether the EPA, in the Agency background document developed for this meeting, had made an appropriate choice of a 3x versus the presumptive 10x FQPA safety factor, and the rational for its decision. Nonetheless, discussion of this point arose several times during the two-day Panel session. Given the importance of the issue, an attempt is made here to summarize the views expressed by Panel members along with the logic behind these views. The Panel recognizes that it is constituted as a technical advisory body, not a group intended to provide legal/policy advice. However the choice to apply particular FQPA safety factors in EPA's cumulative risk analysis clearly involves both policy and science. A legal/policy interpretation is needed to define the standard of evidence required to depart from the mandated default 10-fold factor in any ultimate risk management decisions that might be made on the basis of the cumulative risk analysis. Technical judgments are also needed in assessing whether any particular standard of evidence has been met by the data available for individual organophosphate pesticides or AChE inhibitors as a common mechanism group. The discussion below summarizes the Panel's assessment of the scientific evidence pertaining to the FQPA safety factor.

A majority of the Panel members who commented on the Agency decision of an appropriate FQPA safety factor disagreed with the Agency's proposal to deal with the FQPA requirements to ensure protection of infants and children by selective application of a 3X safety factor. These Panel members concluded that the confidence with the available data was not sufficient to assure adequate protection with less than the 10x FQPA safety factor. Other Panel members were prepared to accept the EPA proposal, some with certain reservations.

The Agency has proposed not to apply the full 10x FQPA safety factor in cases where animal studies have indicated that younger animals (rats) are no more sensitive than adults to AChE inhibition by repeated (as opposed to single dose) exposure to OPs. Where there are data that indicate no greater sensitivity for cholinergic inhibition in weanling animals than in adults, the Agency would apply no special, additional safety factor. The Agency proposes to apply a 3X safety factor (described as a database uncertainty factor) in cases of chemicals that have been shown to be about three-fold more potent as AChE inhibitors in weanling rats than in adults. The Agency also proposes to apply the same safety factor to the 24 remaining chemicals currently under review, while awaiting receipt of new data from ongoing studies of developmental neurotoxicity in rats.

Various reasons were cited by the Panel members who recommended instead that the EPA apply across the board a uniform 10X FQPA safety factor. The most widely cited reason for this recommendation was a concern that the existing animal database does not provide sufficient assurance that young children are not at substantially greater risk than adults from exposures to OPs. This concern was based on the uncertainties arising from several deficiencies in the EPA's cumulative risk analysis. These deficiencies include the following:

1. Extrapolation from data on a limited set of compounds.

The EPA's proposal to use a 3-fold factor for the cumulative risk assessment is based on relative sensitivity to cholinesterase inhibition from a set of six organophosphorus toxicants. At most, an approximate 3-fold difference in sensitivity to cholinesterase inhibition was noted in younger animals following repeated dosing. The EPA considered that this subset of compounds represents the range of variability of differential responses for all 30 compounds under consideration. The age-dependence of differences in sensitivity to cholinesterase inhibition by the other 24 OP toxicants is unknown. This data gap alone was felt to make it prudent to accept the 10x default.

2. Uncertainties about the mechanisms of age-dependent sensitivity in young rats and their applicability to human

beings.

Even with the six compounds known to have relatively small age-dependent sensitivity in young rats, the extrapolation to humans is problematic. First, the mechanism of age-dependent sensitivity in rats has not yet been fully elucidated. More important, we lack comprehensive information about the relative biotransformation capacities for OPs in young and adult humans, and about the relative rates of enzyme recovery by de novo synthesis and other mechanisms. Without detailed information of this sort (admittedly difficult to obtain) we cannot be sure that the relatively rapid decrease in OP sensitivity in weanling rats will also apply to children in the critical 1-2 year age group.

3. Limitations of animal models to identify effects of cholinesterase inhibition in children

While the Agency noted that the OP cumulative risk assessment is based on AChE inhibition and cholinergic toxicity, more relevant indications of whether an exposure to OPs are " safe for children'' are needed, specifically behavioral and cognitive measures such as IQ, attention, language function, etc. Much uncertainty is introduced by using AChE inhibition as a surrogate for these endpoints. For example, as was pointed out at the meeting, it is not known whether a given level of AChE inhibition has the same consequences for a young child as for an adult. Information is largely lacking about the sensitivity, specificity and predictive power of AChE inhibition as a marker for neurobehavioral effects of OPs based on current animal models. In addition, such information is also lacking in terms of high quality epidemiological studies of exposure to pesticides to infants and children. Particularly, the lack of long term neurobehavioral studies at any stage of development creates a great deal on uncertainty in trying to identify the risks of the OPs to children.

4. Uncertainties about the potential frequency of "high-level exposure".

Another consideration in the application of the FQPA safety factor is confidence in the extent to which the exposure assessment truly captures high-end exposures, particularly in children. One Panel member pointed out that although the Agency proposes to consider upper percentile estimates of exposure in the cumulative risk assessment, these estimates may not be as high as the percentiles imply. As evidence for this, an example was cited in which consumption of small amounts of a single food item (e.g., apple or pear) containing a single OP at the upper end of its PDP range could result in exposure above the 95th percentile for cumulative dietary exposure calculated by the Agency. In view of this, an argument could be made for an additional FQPA safety factor if the benchmark for risk management decision is a percentile of exposure that does not adequately address infrequent, but not truly rare, exposure events.

While aware of all these issues, other Panel members nonetheless considered that the Agency's proposal for a 3X safety factor was reasonable, with certain provisions. The major provision asked for by some of these panel members was to use 3X safety factors even for agents that showed no age-dependent sensitivity in rats and an increase to 10X in the case of agents that have not yet been evaluated for potential age-dependent sensitivity. This position was based on a reasonable level of confidence in the existing animal database for the six different OP anticholinesterases so far evaluated for age-dependent sensitivity. This database showed no compounds with more than 3X greater potency in weanling than in adult rats, and several that show identical potency in these two age groups (e.g., methamidophos). Reasonable confidence was expressed that the animal data can be extrapolated to humans in light of the recent data that illuminate the mechanisms underlying age-dependent sensitivity to OP anticholinesterases in the rat. These data demonstrate that at least a large portion of the age dependent sensitivity reflects the maturation profiles of enzymes involved in metabolism and elimination of such agents. Although comparative information on humans is not complete, the species extrapolation is strengthened by information on A-esterase maturation indicating similarly rapid maturation during the period equivalent to early infancy, with near

adult levels reached by the time of weaning in rats in humans. Finally, one Panel member noted that many of the agents in question have been in use for decades and yet, despite isolated cases of acute toxicity, no clear evidence of developmental abnormalities has emerged.

DETAILED RESPONSE TO THE AGENCY'S CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document, dated June 3, 2002, and are presented as follows:

Issue 1. Role of Cholinesterases and Acetylcholine

As discussed in the EPA report, inhibition of acetylcholinesterase (AChE) in the young can result in cholinergic toxicity as in the adult, but evidence has also been emerging over the last several years that indicates that AChE and acetylcholine may serve as neuromodulators in development.

Question 1.1

Please comment on the extent to which the report adequately summarizes the current state of knowledge. Does the scientific evidence support the conclusion that perturbation of the cholinergic nervous system during development by inhibiting AChE can potentially lead to deficits in the structure and function of the central and peripheral nervous systems?

As discussed in the EPA report, inhibition of acetylcholinesterase (AChE) in the young can result in cholinergic toxicity as in the adult, but evidence has also been emerging over the last several years to indicate that AChE and acetylcholine (ACh) may serve as neuromodulators in development.

The Panel concluded that there is a significant potential that brain development could be affected by any agent that blocks the activity of AChE and raises the level of synaptic (or non-synaptic) acetylcholine. Thus the Panel agreed that the scientific evidence support the conclusion that perturbation of the cholinergic nervous system during development, by inhibiting AChE, could potentially lead to deficits in the structure and function of the central and peripheral nervous systems.

Overview

The Panel commends the Agency on the preparation of the section of the report dealing with the potential role(s) of organophosphate (OP) inhibitors on the structure and function of the developing nervous system. Section II A of the report presents information regarding the roles of acetylcholine and AChE in neurodevelopment. It is well known that inhibition of AChE catalytic function leads to the accumulation of acetylcholine, which in addition to its role in cholinergic transmission, also participates in the structural development of neurons. Compelling evidence demonstrates that AChE is a multifunctional protein with a catalytic domain and a surface adhesive domain that may be important for morphogenesis in the nervous system. In vitro studies in which the adhesive site is perturbed have clearly demonstrated a direct developmental role for this domain in both the central and peripheral nervous systems. Finally, inhibition of AChE in the adult leads to the expression of a novel AChE isoform (AChE-R) which has a different tissue distribution from the normal synaptic form (AChE-S) and may serve different functions. Thus, the potential effect(s) of OP inhibitors on the developing nervous system are complex. An elaboration of the Panel's position is provided below.

Elevated acetylcholine levels and neuronal development

The cumulative risk assessment of OP anti-AChEs is based on their common mechanism of toxicity, i.e., phosphorylation of AChE leading to accumulation of acetylcholine and consequent cholinergic signs of toxicity. Importantly, acetylcholine is itself a neuromodulator. Thus, the elevated levels of acetylcholine, subsequent to AChE inhibition, might disrupt neurodevelopment by affecting axonal outgrowth and guidance (Coronas, et al., 2000; Wessler, et al., 1998). The published data provide ample evidence that acetylcholine modulates neural growth and plasticity in addition to its well-known role in interneuronal, neuromuscular and neuroglandular signal transmission. Acetylcholine effects are mediated by diverse subtypes of ionotropic and metabotropic receptors. Inhibition of synaptic AChE by OPs causes excessive activation of nicotinic and muscarinic receptors. The former responds by rapid conformational change to an inactive, desensitized state. On the other hand, muscarinic receptors respond by down regulation (i.e. their numbers are reduced). The functional impact of such changes on the developing brain would be very serious if they were prolonged, for example, if AChE activity does not recover.

Eskenazi and co-workers (1999) recently reviewed the evidence that repeated low-level exposure of animals to OP pesticides might affect neurodevelopment and growth in developing animals. For example, animal studies have reported neurobehavioral effects such as impairment on maze performance, locomotion, and balance in neonates exposed in utero and during early postnatal periods. Possible mechanisms for these effects include inhibition of brain AChE, down-regulation of muscarinic receptors, decreased brain DNA synthesis, and reduced brain weight in the offspring. Research findings also suggest that it is biologically plausible that OP exposure may cause dysregulation of the autonomic nervous system. Downstream effects at multiple sites, including the lungs, could predispose children to a variety of disabilities. All such changes can be considered endpoints elicited by the common mechanism of toxicity and must be anticipated from exposure to any OP anticholinesterase.

Another downstream effect that could potentially result from the common mechanism of OP toxicity is the compensatory upregulation of novel forms of AChE that do not function quite like the normal forms. Some recent research suggests that inhibition of AChE in adults stimulates production of an AChE variant known as “read-through” AChE (because the normal transcriptional splicing at the C-terminus is omitted). The major AChE expressed in nervous tissue is the so-called “synaptic” form. Chronic inhibition of AChE activity can lead to the expression of the unique “read-through” product, which is secreted as a monomer (Grisaru, et al., 1999; Soreq, H. and S. Seidman, 2001). This protein has the same enzyme kinetics as the synaptic form and thus would behave like other forms of AChE in a typical assay. However, because read-through AChE has a different distribution within the cell, and from tissue to tissue, it may not have the same functional impact as normal AChE. The presence of read-through AChE has not yet been described in the fetus or the neonate, nor has there been any study of the potential for developmentally significant modulation of this form after OP exposure. Nonetheless, the possibility of such effects, or additional changes in protein expression that may eventually be revealed by proteomic studies, reinforces concerns that OPs might exert developmental neurotoxicity through their common mode of action. These observations also give rise to a concern that apparent recovery of assayed total brain cholinesterase following OP inhibition might not indicate a return to a completely normal state in a developing nervous system.

Direct role for AChE in development

There is also evidence that AChE inhibitors could disturb neuronal development by mechanisms in addition to the common mode of action. AChE is developmentally expressed by neurons during axonal outgrowth and migration, periods when its role in terminating cholinergic transmission would be unnecessary (Drews, U. 1975; Grisaru, et al., 1999; Layer, P.G. and E. Willbold. 1995; Soreq, H. and S. Seidman. 2001). Experimental studies in vitro, involving perturbation of AChE either by certain non-OP AChE inhibitors or AChE-specific antibodies, confirm a specific developmental role for AChE (Bigbee, et al., 1999; Dupree and Bigbee. 1994; Layer, P.G., et al., 1993). In addition, observations by Slotkin and collaborators have demonstrated persistent neurobehavioral and DNA/protein abnormalities in rats subjected to moderate or low dose AChE inhibitor treatment in utero or in an early postnatal

period. A direct role for AChE in the process of neural development has also been demonstrated by genetic manipulation of AChE expression, either by stable transfection or by antisense treatment (Bigbee, et al., 2000; Brimijoin, S. and C. Koenigsberger. 1999; Grisaru, et al., 1999; Sternfeld, et al., 1998).

In tissue culture, AChE that is catalytically inactivated by point mutation of the active site serine can still support some morphogenic phenomena (Sternfeld, et al., 1998). Such findings indicate that the morphogenic potential of this enzyme is at least partially independent of its esterase activity, possibly because of morphogenic properties in the adhesive domain surrounding the opening of the active site gorge. Results from studies using transgenic mice, however, have produced results that raise questions about the significance of adhesion-based functions of AChE in brain development. In one study, it was shown that neuronal development and structure of the brain are apparently normal in AChE knockout heterozygote mice that have only 50% of normal AChE expression levels (Xie et al. 2000). Even complete AChE knockout causes no profound changes in the structure of cholinergic pathways in the brain as revealed by histochemistry and immunohistochemistry (Mesulam et al., 2002).

It can also be questioned whether OPs are likely to influence the adhesive functions of AChE, in contrast to certain long-chain, bis-quaternary AChE inhibitors that bind reversibly to catalytic and peripheral sites. No evidence exists to indicate that OPs bind to the adhesive domain. On the other hand, as pointed out in the EPA report, the possibility exists that an OP could alter the three-dimensional structure of AChE by binding to the active site, thereby subtly altering the surface adhesive domain. For this reason, as was pointed out by one Panel member, there is need for additional pharmacodynamic studies to better define the different OPs and their structural interactions with AChE.

At present, it would be prudent to recognize the potential for developmental toxicity stemming from mechanisms that operate in addition to the common mode of OP toxicity. Thus we must recognize that the degree of AChE inhibition may not fully capture the ability of an OP to perturb the development of the nervous system. AChE is uniquely high during critical periods of development and thus may be especially vulnerable for short periods. Furthermore, a given degree of inhibition of AChE in the fetus or neonate may have a greater effect than the same level of inhibition in the adult. However, the current review is almost completely qualitative. There is no quantitative analysis relating either the presence or the extent of developmental effects and duration of measured or estimated cholinesterase inhibition in the developing brain. This quantitative component is a key missing link in the chain of analysis that is needed to assess whether the degree of cholinesterase inhibition that has been judged statistically detectable for adults should also be expected to be without appreciable consequence during development.

The Panel generally agreed that the existing evidence falls short of what would be needed to prove that AChE inhibition during development will cause later deficits in nervous system structure and function. However, while definitive evidence is lacking, the potential nevertheless exists. Of particular importance to the risk assessment of OP toxicants, more recent information suggests that some OP inhibitors of AChE can modify neuronal growth in vitro. It should be stressed, however (as noted in the Report) that some anticholinesterases have no apparent effect on neurite outgrowth. Some studies suggest that neurodevelopment may be affected in vivo by some OP toxicants, but most of these studies utilize unrealistic exposure conditions and thus have uncertain relevance for risk assessment. In addition, there could be very subtle changes not disclosed by standard behavioral tasks. The two best-described systems are both sensory in nature and are difficult to assess. The report did not summarize the studies for neuro-behavioral effects from fetal exposure to the OPs. Thus it is difficult to determine whether there is a common potential for neurological and behavioral effects. This issue needs greater clarification in the document, especially as it relates to registrant-provided developmental neurotoxicity (DNT) studies. Without this comprehensive review and evaluation of a larger number of DNT studies, it is difficult to assess whether the existing data support or refute a common, additional developmental risk above the adult risk, for a common level of AChE

inhibition.

Issue 2. Age-Dependent Sensitivity to Cholinesterase inhibition in Animal Studies

Age-dependent sensitivity (*i.e.*, young animals can exhibit higher levels of cholinesterase (ChE) inhibition at the same dose or inhibition at lower doses compared to adults) has been observed in several laboratory studies following treatment (acute and/or repeated oral gavage doses) of neonatal, juvenile, and adult rats with organophosphorus (OP) pesticides. The exact mechanisms of this age-dependent sensitivity are not known, but several studies have demonstrated that toxicokinetic factors may be responsible. Most notably, the more limited ability of the young to detoxify OP pesticides by A-esterases and carboxylesterases appears to be an important factor underlying the increased sensitivity of the immature rat to ChE inhibition. There appears to be more rapid recovery of inhibited AChE (synthesis of new ChE enzyme) in postnatal (and fetal) rat tissues, but information on comparative recovery in children and human adults is lacking.

Question 2.1

Please comment on the extent to which the report adequately discussed and summarized the current understanding of age-dependent sensitivity to ChE inhibition, the prevailing views in the scientific community concerning the biological factors involved, and the role esterases may play as a major factor accounting for potential increased sensitivity of the immature rat.

Age-dependent sensitivity (*i.e.*, young animals can exhibit higher levels of cholinesterase (ChE) inhibition at the same dose, or inhibition at lower doses compared to adults) has been observed in several laboratory studies following treatment (acute and/or repeated oral gavage doses) of neonatal, juvenile, and adult rats with organophosphorus (OP) pesticides. The exact mechanisms of this age-dependent sensitivity are not known, but several studies have demonstrated that toxicokinetic factors may be responsible. Most notably, the more limited ability of the young to detoxify OP pesticides by A-esterases and carboxylesterases appears to be an important factor underlying the increased sensitivity of the immature rat to ChE inhibition. There also appears to be more rapid recovery of inhibited AChE (synthesis of new ChE enzyme) in postnatal (and fetal) rat tissues, but information on comparative recovery in children and human adults is lacking.

The Panel considered the Agency's summarization of the current literature to be adequate in some areas and deficient in others. The discussion and summation of the age-dependent toxicity of the six OP insecticides for which data are available was concise and complete, and Tables 1 and 2 were helpful and informative. Toxicokinetic factors were proposed to be critically important in age-related sensitivity and limited discussion was provided in the document on the detoxifying esterases (carboxylesterase and A-esterase) and their differential expression during maturation. However, little is mentioned regarding differences in oxidative metabolism and its potential role in differential sensitivity via differential rates of metabolism of some OPs to more active forms. The report documented the role of A and B esterases in the limitation of AChE inhibitor action and the importance of AChE resynthesis as a means of differential recovery from enzyme inhibition. However, the discussion of the biological factors [specifically, A-esterases and carboxylesterases] that might result in age-dependent susceptibility to toxicity of certain OPs could be significantly improved by presenting a more balanced interpretation of the available data.

Some anticholinesterases show distinct age-related differences in effects, while other OP agents appear to express little age-related differences. Differences in sensitivity tend to be smaller with repeated dosing and may also be a function of age of the developing animal. Several factors may contribute to this finding, including faster recovery of acetylcholinesterase in tissues of young animals and increasing levels of detoxifying esterases with

increasing maturity of metabolic systems. Several studies have shown that the sensitivity of the target enzyme in tissues from different age groups does not differ. Thus, sensitivity of acetylcholinesterase molecules themselves probably does not contribute to age-related sensitivity. Differences in cholinergic receptor adaptation were also considered. Cholinergic receptors often downregulate following cholinesterase inhibition, but differences in receptor adaptation do not appear responsible for age-related sensitivity. The Agency's background document also mentions the presence of muscarinic autoreceptors, capable of inhibiting acetylcholine release presynaptically. In fact, the postnatal maturation of the muscarinic autoreceptor correlates roughly with decreasing acute sensitivity to OP toxicants and may therefore play a role in age-related sensitivity.

The Agency's background document summarized evidence that supports important roles for A-esterases and carboxylesterases in the increased sensitivity of the immature rat, but ignores observations or interpretations that might suggest other possibilities. Consequently the document tends to overstate the degree to which the mechanisms of age-dependent toxicity of OPs are understood. This is most apparent with regard to three issues:

1. The document summarizes several studies that have reported correlation between the temporal patterns of development of A-esterase and carboxylesterase activities and OP sensitivity. However, the document does not mention that some of those studies also have reported a decreased capacity of immature rats to oxidatively activate these same insecticides. Immature rats have reduced A-esterase and carboxylesterase activities, but they also have a similarly reduced capacity to produce the oxygen analogs from the parent insecticides. This is an important potentially offsetting observation that should be discussed in the report. It should be noted that no targeted mechanistic studies have evaluated the role of these esterases in age-related sensitivity. Thus, only a correlation between inherent esterase activity levels and sensitivity to the anticholinesterases support the concept of esterase-mediated differential sensitivity.

2. The report presents evidence in support of a role for A-esterase in detoxification of certain OPs and in age-dependent sensitivity, but does not discuss evidence that might be contrary to this view. There are only three oxons that have been identified that are substrates for A-esterase *in vitro* – paraoxon, chlorpyrifos oxon, and diazoxon. Studies with knockout mice have indicated that paraoxon metabolism by A-esterase is probably insignificant *in vivo*. And as indicated in the document, knockout mice were much more sensitive to chlorpyrifos oxon or diazoxon. However, not mentioned in the document was the observation that knockout mice were only slightly more sensitive to the parent compounds chlorpyrifos and diazinon, and even then only at high doses, suggesting that A-esterase may not be an important detoxification pathway upon exposure to the parent insecticides. In addition, some reports in the literature have suggested that A-esterase in the rat probably only plays a role in detoxification when the chlorpyrifos or diazinon doses are very large. At small to moderate doses, detoxification by A-esterase is probably insignificant compared to detoxification through carboxylesterase. The report should include some discussion of this issue.

3. The document, referring to Table 2 on p. 22, states that the temporal pattern of A-esterase and carboxylesterase activities correlate reasonably well with studies on OP sensitivity. But it does not discuss possible exceptions to this correlation. For example, methyl paraoxon is not a substrate for A-esterase, and has limited interactions with carboxylesterase. Therefore, one should expect limited age-dependent sensitivity yet its acute age-dependent sensitivity (from Table 1) is almost the same as that of chlorpyrifos, and its age-dependent toxicity after repeated administration might even exceed that of chlorpyrifos (again from Table 1). These observations could suggest involvement of other factors in the age-dependent sensitivity of at least methyl parathion.

The discussion focused on developmental profiles of esterases exclusively and ignores changes in cytochrome P450 activities with age as a potential contributing toxicokinetic factor in age-related sensitivity to OPs. The discussion should be expanded to include a description of the state of knowledge on P450 development in the rat, focusing primarily on isoforms known or suspected to be involved with OP bioactivation and detoxification.

The Agency needs to include a discussion in the background document on the implications of different possible dose metrics in explaining age-related sensitivities through metabolism. There should be a clear articulation of reasonable alternative hypotheses about which dose metric(s) for ChE could be important for the developmental pharmacodynamic actions of anti-cholinesterase agents. For example, it is possible that the best dose metric for predicting effects could be "peak" levels of cholinesterase inhibition on one day or several days of successive exposure. Alternatively, an "AUC" measure of the integral of % inhibition X time could prove to be the closest causally relevant predictor of developmental effects. There are also a few more complex hypotheses. In any event, given each of these and/or other plausible measures of internal delivered "dose", a discussion should be included on the roles of activating vs. detoxifying enzyme activities and other factors in this context. As an example, for measures of acute peak cholinesterase inhibition by OPs requiring activation for biological activity, activating enzyme activities will be important and detoxifying enzymes such as the esterases will tend to be less important. The opposite would tend to be the case if AUC (integrated % inhibition X time) over an extended period of dosing is more important for causing developmental effects—in that case, activating activity would be somewhat less important and detoxifying enzyme activities for both the parent chemical and the activated intermediate would tend to be more important.

Question 2.2

Please comment on the timing of administration (i.e., the developmental stage treated) and the differential found between adults and the young animal.

The Panel interpreted the question as a query on the impact of dosing parameters on relative sensitivity of different age groups. The Panel concluded that the timing of exposures is critically important in evaluation of age-related differences in sensitivity to anticholinesterases. The Agency's background document describes a number of studies, some with prenatal, some with postnatal, and some with combined prenatal/postnatal exposures. Based on cholinesterase inhibition, the studies utilizing exclusively prenatal dosing appear to report consistently equal or lesser effects in the developing organism than in the dam. This may in some cases be due to the timing of biochemical measurements relative to exposure, but the findings generally suggest no higher sensitivity to cholinesterase inhibition in prenatally-exposed animals. The reverse is often found when animals are exclusively treated postnatally. In essence, higher toxicity and more extensive cholinesterase inhibition are often noted in neonatal animals compared to older immature animals, and even greater differences in sensitivity arise when comparing very young animals to adults dosed similarly with a number of OP toxicants. With acute, relatively high exposures, several OP insecticides are markedly more toxic to very young individuals. This kind of stage-related sensitivity is compound specific and it appears to be directly related to the maturational state of A-esterases and carboxylesterases. Compounds that are not substrates for one or both of these developmentally regulated enzymes appear generally not to show differential inhibition based on timing of bolus injection. In some cases, however, age-related sensitivity may occur with OP toxicants that are not well detoxified by either carboxylesterase or A-esterase (e.g., young animals are markedly more sensitive to methyl parathion). Other toxicokinetic or toxicodynamic factors may therefore play an important role in age-related sensitivity.

In contrast, when immature and adult rats were repeatedly exposed to some OP insecticides (e.g., chlorpyrifos), relatively little age-related differences in cholinergic toxicity were noted. The ability to recover between exposures in tissues from younger animals may be important in this regard, i.e., if AChE molecules are being

synthesized faster in immature animals, overall enzymatic activity will recover faster following each cholinesterase inhibitor exposure, thereby reducing accumulation of insult. Because of the relatively short maturation period in rodents, however, repeated dosing studies can change the baseline, i.e., the animal is becoming less sensitive to the pesticide throughout the dosing period. Thus, lesser age-related differences in sensitivity with repeated, compared to acute exposures, may be due both to inherent differences in recovery potential and to decreased sensitivity as the dosing period progresses. One could question if changes in enzyme recovery may even cause a reversal of age-related sensitivity in repeated dosing paradigms; that is a situation in which adults are more sensitive than younger animals. In fact, several studies (Chakraborti *et al*., 1993; Pope and Liu, 1997; Zheng *et al*., 2000) suggest that while neonatal rats are markedly more sensitive to acute exposures to chlorpyrifos, fewer differences are noted with daily dosing, and if intermittent dosing (every four days) is used, more extensive neurochemical changes (i.e., AChE inhibition, muscarinic receptor downregulation) may occur in adults. These findings imply that more rapid recovery of AChE activity noted in the immature animal's brain following OP exposure can in fact contribute to more rapid functional recovery of neurotransmission.

Question 2.3

Please comment on the extent to which comparative ChE data on six OP pesticides (chlorpyrifos, diazinon, dimethoate, methamidophos, malathion, methyl parathion) may represent a reasonable subset of different structural and pharmacokinetic characteristics of the cumulative group of OP pesticides to define an upper bound on the differential sensitivity that may be expected at different life stages of the immature animal. As an example, there are no chemical-specific comparative cholinesterase data on azinphos-methyl (AZM), an important contributor of risk for the food pathway. Pesticide-specific comparative cholinesterase data on the other six pesticides from the OP class (including data on malathion, a member of the same chemical subgroup as AZM) show a limited range of differential sensitivities -- from one-fold (no increased sensitivity) up to three-fold -- between the young and adults. EPA regards these data on other OPs as providing sufficient evidence to assess the potential for AZM to show age-dependent sensitivity, and to reasonably predict the degree of potential difference in sensitivity between the young and adults. Given the results of the other OPs, EPA concludes that it is unlikely that AZM would exceed a magnitude of difference greater than approximately 3-fold following treatment of PND 11 through 21 pups versus adult animals.

The majority of the Panel members concluded that the comparative data on six OP pesticides (chlorpyrifos, diazinon, dimethoate, methamidophos, malathion, and methyl parathion) should not be considered to represent a reasonable subset of different structural and pharmacokinetic characteristics of the cumulative group of OP pesticides to define an upper bound on the differential sensitivity that may be expected at different life stages of the immature animal. However one Panelist dissented from this view, and agreed with the report that these six pesticides could be used to define an upper bound on the differential sensitivity for the cumulative group.

Specific comments by Panelists against the use of the 6 OPs as a representative subset of the cumulative group were as follows:

The currently available data on direct postnatal exposure of six OP pesticides shed some light on the potential differential sensitivity of OPs during stages of development. The Agency is to be commended for the extensive effort in addressing these rather complicated issues. However, the complex interplay of many factors (e.g., pharmacokinetics and pharmacodynamics that are chemical- and developmental stage-specific) leading up to the inhibition of brain ChE inhibition is the source of substantial uncertainty for predicting the upper bound of the differential sensitivity for all the OPs under evaluation.

The document suggests that the age-related change in sensitivity to certain OPs is largely a function of toxicokinetic

factors since age-related changes in acetylcholinesterase catalysis and sensitivity to inhibitors do not occur. If this is the case, one must consider whether or not the toxicokinetic characteristics of any remaining members of the cumulative assessment group are sufficiently different from the six indicated in the document, so as to lead to a juvenile/adult differential toxicity greater than a 3-fold uncertainty factor. Based on the lack of information in the open literature regarding the toxicokinetic characteristics of the remaining pesticides (most importantly their metabolism and volumes of distribution), one must conclude that there simply is not enough information available to know whether or not the six insecticides indicated in the document are representative toxicokinetically of the cumulative group. Consequently, we do not know if those six OPs can define an upper bound for the possible differential age-dependent sensitivity of other OPs.

One Panelist offered differences in potency among ChE agents as an illustration of the uncertainties involved in extrapolating biological properties between agents. A more that 10-fold difference in the relative potency factor (RPF) is observed between the metabolic activation pair of acephate and methamidophos, just within adult female rats. For these two chemicals, and with the rich database available for methamidophos, the Agency's document stated that it is not possible to determine "whether acephate would show comparable responses in adult and young rats" (page 13, *Determination of the Appropriate FQPA Safety Factor(s) in the Organophosphorus Pesticide Cumulative Risk Assessment*; June 10, 2002). Other than obtaining chemical-specific data, much more information is needed for a reliable estimate of a range of age-related sensitivity of OPs. There are insufficient data to fully support a 3-fold uncertainty factor based on an estimated upper bound of 3-fold age-related differential sensitivity.

It should also be noted that dose-response modeling would give a more consistent comparison for the age-related sensitivity among chemicals, and the Agency's analysis showed that the upper bound would be 4-fold based on data for methyl parathion. Presumably this is only based on the data from repeated dosing, and not including the single dosing study that showed up to 7-fold differences. Thus, given the current data, it may be prudent to consider an upper bound of greater than 3 just for the toxicity side of the uncertainty factor consideration.

Overall, it is ill-advised to speak of an "upper bound" from the six available observations in this case. "Upper bound" conveys the impression of a firm, known upper limit and the existing data cannot support a conclusion of this sort with any reasonable degree of confidence. It is even challenging to attempt a distributional treatment from such a small number of chemicals but this is the best treatment that can be made. A first step should be to apply either the Agency's exponential model as presented at the February 2002 SAP meeting, or, where the data are insufficient for this, a simplified version of it to express the apparent relative potency based on estimated ED10's of the chemicals for either acute or repeated dosing exposures for animals of various young age groups versus adults. The simplified exponential model is needed because some of the current calculations distort the relative potency of the cholinesterase inhibition results in young versus adult animals by failing to take into account the fact that no more than 100% of the enzyme can be inhibited. For example, the calculation from the Moser et al acute dosing data for male animals is based on a simple ratio of 89% inhibition in pups versus 39% inhibition in adults. Clearly, with a simple ratio, even if the true potency ratio in the two groups were 100 or 1000, the calculation could not produce a result larger than 100/39 or approximately 2.5. The 2.3 in the document becomes about 5 when one applies a simple one-parameter version of the exponential model. One Panelist suggested a revised experimental model, as presented by the Agency, that uses a basic exponential form, but omits the high dose saturation level of inhibition and the expanded model's low dose nonlinearity feature:

$$\text{Fraction inhibited} = 1 - e^{-kd} \qquad (1)$$

Where d is the dose and k is the measure of potency (inhibition units per dose at low doses). This model at least corrects for the fact that one cannot get more than 100% inhibition while calculating apparent potency in each group.

Using this simplified exponential model, the relative potency for two comparable experiments in animals of

different age is just the ratio of k_1 for the younger age group to k_2 for the older/adult age group, or:
Potency in young age group relative to adults (k_1/k_2) =

$$\frac{d_{2\ (\text{adult animals})}\ln(1 - \text{Fraction inhibited in young})}{d_{1\ (\text{young animals})}\ln(1 - \text{Fraction inhibited in adults})}$$

(Alternatively, one could use equation 1 to estimate ED10's for each group and take a ratio of the ED10's as the measure of relative potency. The results of this would be very similar to the ratio of "k" potency factors described above).

This equation for relative potency in adult and young animals incorporates a saturation at 100% cholinesterase inhibition and also corrects for the situation where the inhibition findings are from different doses. Putting in an upper limit of inhibition short of 100% (as is found necessary in some cases in the Agency's modeling) would tend to increase the pup/adult sensitivity ratios in cases where the pup shows greater inhibition than the adult.

A particular challenge for this proposed analysis applies to cases such as malathion where in some cases there is no detectable cholinesterase inhibition in adult animals at rather high doses, but there is appreciable inhibition at comparable and lower doses in younger animals. Simply excluding these cases risks biasing the analysis, so some truncated distributional analysis is needed here.

Other specific comments offered by one Panelist in support of the use of the 6 OPs as a representative subgroup of the cumulative risk were as follows:

First, there is no inherent difference in the ChE enzymes or its binding to an OP between young and adult animals. Second, the difference between inhibition of ChE between newborn, pups and adult animals is primarily due to two factors, which are the rate of regeneration of the enzyme and the level of various enzymes, such as the esterases and others, that detoxify the OP, neither of which will be different among the compounds that are tested. The main difference among the test compounds is going to be the relative rate of detoxification.

In general, the 6 OPs for which data are available for ChE inhibition of young and adult animals are qualitatively similar with respect to ChE inhibition. For these compounds, the ratio of ChE inhibition of adult to pup sensitivity ranged from no difference to three fold. Based on this information, the Agency has included a 3-fold uncertainty factor. The 3-fold factor is reasonable since the range of 1 to 3 fold is based on dosing of large amounts of OPs directly to the pup and adult animals, which represents exaggerated exposure conditions. Under more realistic conditions of exposure to pregnant or lactating dams, the degree of inhibition in the neonates and the pups was generally less than the dam.

Overall, the prediction of the range of enzyme inhibition is more limited than the prediction of toxicity and the lack of information for the other OPs and the uncertainty in making this estimate is taken into account by the incorporation of a 3-fold uncertainty factor.

Issue 3. Relevance of the Animal Findings to Children

Age dependent sensitivity to cholinesterase inhibition has been associated with the limited ability of the immature rat to detoxify OP pesticides by esterases. In rats, A-esterase activity increases from birth to reach adult levels around postnatal day 21. Fetal rats possess very little carboxylesterase activity with increasing activity as the

postnatal rat matures, reaching adult values after puberty (50 days of age). Data showing increased sensitivity of the young animal to cholinesterase inhibition compared to adults has generally been derived from acute dosing of PND 7 or PND 11 pups, or repeated dosing of PND 11 to PND 21 pups. The available data also show as the young rat rapidly matures in its ability to detoxify by esterases, the differential in cholinesterase inhibition becomes smaller. Thus, the relative sensitivities of immature rats found in the studies of dosing pups through PND 11 to 21 are smaller compared to studies of dosing a PND 11 pup. The dosing studies of PND 11 through 21 pups are considered to better approximate the maturation profile of the A-esterases of the highly exposed children's age group in the OP cumulative risk assessment, the one and two year olds, compared to a study of a PND 11 pup which is similar to a newborn. Thus, the repeated rat dosing studies more closely mimic the maturation or developmental profile of A-esterase appearance in children around the one and two year olds where children are reaching adult levels of A-esterase activity. The use of dosing studies of PND 11 through 21 is consistent with the exposure patterns of children. Humans generally do not begin to consume fresh (uncooked) fruits and vegetables until after six months of age or more. Furthermore, repeated dosing studies were used to determine relative sensitivity because people are exposed every day to an OP pesticide through food, and thus an animal study using repeat exposures is considered appropriate. Finally, following exposure to an OP, regeneration of cholinesterase to pre-exposure levels does not occur for days or weeks, making the exposed individual potentially more vulnerable to subsequent exposures during that period.

Question 3.1

Please comment on the maturation profile of A-esterase and the uncertainties surrounding these data in young children. Because no human data are available on the maturation profile of carboxylesterases, please comment on what should be assumed in humans, especially children age 1 to 2 years, given the animal data and what science understands in general about detoxification maturation profiles.

The Panel concluded that there is appreciable residual uncertainty about the differences in activity at early versus adult life stages in relevant activation and detoxification pathways in animals and humans, especially for detoxification by carboxylesterases.

Many Panel members provided generally similar perspectives. The discussion below begins with evaluations of the specific data cited in the Agency's background document for the changes in A-esterase levels during development. With this as background, the Panel responded to the last part of the question with a review of the general profile of changes in whole-body elimination half lives for drugs in general, and drugs eliminated by various specific pathways. In the absence of more direct evidence for developmental changes in carboxylesterases and other even less well characterized routes of elimination, these data provide the most applicable starting point for defining baseline expectations and associated uncertainties.

Specific Data on Changes in A-esterases and P450 Activating and Detoxifying Enzymes During Development

It would be useful to include more information in the Agency's background document on metabolic enzymes and metabolism since the rate of detoxification appears to contribute to the differences in the relative inhibition of ChE at various ages as compared to the adult in rats. Carboxylesterases and A-esterases have been shown to be important in the detoxification of some OP toxicants in rats, and may contribute to age-related differences in sensitivity in humans. However, some studies suggest that other metabolic factors may also be important contributors to age-related sensitivity for other OP agents. The entire spectrum of enzymes responsible for activation/detoxification of the OP toxicants should be evaluated for potential changes in enzyme expression and function during human development and their potential contributions to relative sensitivity. Determination of activities of all processes in human tissues would be ideal, but difficult to accomplish. Additionally, while the

relative contributions of blood and tissue detoxification can be estimated in animal models, this information is unknown in humans for most if not all OP toxicants. This subject therefore represents a potentially significant uncertainty in how young children may respond to OP toxicants relative to adults based on differential metabolism.

Both the carboxyesterases and A-esterase are non-specific esterases. Data are available concerning changes in the levels of A-esterase in blood with age in humans, which are about 20 % of adult levels at birth and near adult levels by 6 months of age; however, there are fewer data for the carboxylesterases during development. Several Panel members felt that data should be collected at least with blood carboxylesterases to limit the uncertainty associated with that missing information. There is a complete lack of data about the activity levels of these esterases in the liver and other tissues where the bulk of the detoxification is likely to occur.

At birth, the esterases in general, like many other enzymes responsible for metabolism, are at a low level—approximately 20% of adult values. These enzymes increase rapidly during the first few months and although variable, are near the adult level (60-70%) at six months. The fact that the OP exposure of very young infants is estimated to be smaller than that of other age groups tends to reduce concerns arising from neonatal deficiencies in esterases that detoxify OPs. One Panel member noted that the development of the various esterases appears to be generally similar and the carboxyesterases are likely to be similar to the A-esterases in this regard.

Some Panel members felt strongly that EPA should not accept the remaining data gaps on the relative importance of different esterases for detoxification of different OPs for any length of time. Now that EPA research scientists have developed an in-house assay that at least approximately tracks the age dependent shift in blood samples' ability to alter OP availability *in vitro* based on A-esterase and carboxylesterase activities, these assays should be performed with human blood samples at all ages of interest and with all environmentally relevant OPs. The problem with carboxylesterase is that human blood contains very little of this enzyme, which is largely confined to liver. Therefore, for the foreseeable future, the Agency must continue to reason by analogy with animal data and with the developmental profile of other liver drug metabolizing enzymes. In this context, however, it does appear reasonable to assume that the youngest infants will indeed be deficient in carboxylesterase expression, and that expression of this enzyme will approach adult levels sometime in early childhood—possibly in the 1-2 year bracket.

Some OPs are initially metabolized by cytochrome P450s to oxon intermediates. It appears that the P450s involved are P450 3a and 2D6 families. Cytochrome P4502 D6 expression is decreased in the newborn's liver and then approaches the adult level within a few weeks. Family 3 enzyme overall activity is generally thought to be increased during the newborn, infancy and early childhood stages of life. Family 3a during development is primarily composed of P4503a4 and 3a7. P4503a7 is the fetal form of family 3a and is expressed in high levels in the fetal, newborn and infant liver as compared to the adult. The P450 3a4 is expressed at higher activity levels during these periods than in adulthood. These findings are somewhat substrate dependent and to the Panel's knowledge, studies of the capacity of 3a7 to metabolize OPs have not been conducted. The changing expression of these P450 forms may add to the overall toxicities of the OPs to the human during development. The expression of these enzymes in the human brain during development has not yet been extensively studied.

Detoxification Maturation Profiles

Overall, the pattern of age-related change in the A-esterase bears a close resemblance to general patterns of change for elimination inferred from human observations of age-related changes in the pharmacokinetics of therapeutic drugs. Table 1 reproduces the results of an analysis by Hattis et al. (2002, in press) and Ginsberg et al. (2002). The table shows geometric means ± 1 standard error range of the ratios of the half lives of drugs eliminated by a variety of pathways in children of various age groups relative to adult half lives. Overall, premature infants show on average about a four-fold prolongation of elimination half life for the typical drug; and infants under 2 months of age have about double the half life of adults. The 6 month to 2 year age group shows, if anything, a slightly shorter geometric mean half life than in comparable adult studies. If these patterns hold for activation and

inactivation pathways for OPs, then agents that do not require metabolic activation would be expected to pose greater risks in very young full term infants (achieving comparable blood levels at about half the long term internal dose per mg/kg of external dose) but children in other age groups would, on average show no greater pharmacokinetic sensitivity than adults. Other things being equal, it seems most likely that the unmeasured carboxylesterase will behave similarly, but how confident one should be about this is open to question.

A further topic where data are available is the extent of human inter-individual variability in half lives as a function of age. Variability is much larger than adults in the age groups up until about six months, but reverts approximately to adult levels of pharmacokinetic variability thereafter.

Table I. Geometric Mean Ratios of Child/Adult Elimination Half-Lives. Data Represent Regression Results from 135 Data Groups for 41 Drugs, Log(Arithmetic Mean Half-Life) Data

Major Elimination Pathway							
All pathways							
All CYP (P450 metabolism)							
All Non-CYP							
Unclassified							
more detailed classification:							
CYP1A2							
Renal							
Glucuronidation							
CYP3A							
CYP2C9							
Other, mixed CYP's							
Other Non-CYP's (not renal, glucuronidation)							

[a]Parentheses show the ± 1 standard error range.

R
Question 3.2

Please comment on the extent to which the biological understanding of observed age-dependent sensitivity to cholinesterase inhibition in laboratory animal studies informs our understanding about the likelihood of similar effects occurring in children; in particular, what can be inferred from animal and human information regarding the potential for different age groups to show increased sensitivity if exposed to cholinesterase-inhibiting pesticides.

Does the scientific evidence support the conclusion that infants and children are potentially more sensitive to organophosphorus cholinesterase inhibitors?

The scientific evidence supports the conclusion that infants and children are potentially more sensitive to OP cholinesterase inhibitors than are adults. There are still important unresolved questions including: 1) What is the extent of age-dependency in human fetuses, children, juveniles, adults (and the elderly) and is it larger or smaller than in rats? 2) What are the ages at which higher sensitivity is present in humans as compared with rats (e.g., are 1-2 yr. humans best modeled by the PND 21 rat)? 3) Are underlying mechanisms contributing to age-related sensitivity fundamentally similar? and 4) Does a certain degree of acetylcholinesterase inhibition in the immature system leads to equivalent neurochemical consequences as those observed in adults - or, by contrast, are there likely to be some adverse neurodevelopmental consequences for amounts of brain cholinesterase inhibition that are considered reasonably tolerable by adults?

The understanding of differential age-related toxicity in experimental animals exposed to OP toxicants suggests that, with acute high exposures, young children may be markedly more sensitive to some agents. This is likely based on both toxicodynamic and toxicokinetic factors including differences in expression of detoxifying esterases, possible differences in activation of some agents, and in maturation of adaptive processes that limit or modulate anticholinesterase toxicity. With other OP toxicants (e.g., methamidophos), lesser or even no age-related differences in acute sensitivity may exist. Some studies suggest, however, that differences in sensitivity are less pronounced or non-existent with repeated dosing. Both kinetic (e.g., detoxification) and dynamic (e.g., feedback inhibition of acetylcholine release) pathways are most likely important in contributing to age-related differences in sensitivity to high dose exposures, i.e., these processes are likely challenged only when high levels of the toxicant occur in the system. Thus, with repeated, lower exposures, lesser differences in sensitivity would be expected. As noted above, however, it is likely that the reduced age-related differences with repeated exposures in rodents is due to rapid maturation of the animal with consequent decreased sensitivity over the course of exposure. Therefore, the Panel agreed that the scientific evidence supports the conclusion that infants and children are potentially more sensitive to OP cholinesterase inhibitors to acute high dose exposures. With lower and repeated exposures, the evidence for higher sensitivity in young individuals is not as convincing.

In the absence of directly applicable data, it was felt that humans might differ from rats in the extent and nature of age-dependent sensitivity for enzyme inhibition. All the animal data were generated using either direct exposure to neonates, juvenile and adult animals at very high doses or the treatment of pregnant or lactating animals, also at relatively high dose levels. The data from the repeated direct dosing experiments yielded ChE inhibition sensitivity ratios of 1 to 3-fold for pups versus the adults. This could become as much as 10-fold following acute dosing. Whether this makes a substantial difference in humans likely depends on the exposure level.

As stated above, the remaining data gap regarding human blood A-esterase-mediated detoxification of the different OP anticholinesterases should be addressed by further research. A number of experimental approaches were proposed by Panel members. One was the use of an in vitro model recently developed (Padilla et al., 2002). Now that EPA scientists have developed an in-house assay that at least approximately (and perhaps quite accurately) tracks the age dependent shift in blood esterase abilities to alter OP availability *in vitro*, as a direct comparison between species, these assays should be run with human and rat blood samples at all ages of interest and with all environmentally relevant OPs. For carboxylesterase, a complication exists for projection between species, i.e., in humans (in contrast to rodents), very little of this enzyme is found in the blood. One Panel member recommended a set of studies on the age-related variation in sensitivity of blood cholinesterases to inhibition by OP inhibitors in a primate model, preferably a higher primate. An advantage of a primate model is the similarity in plasma carboxylesterase activity, i.e., primates are deficient in this pathway. Such studies would provide the most relevant

possible animal data on several fronts, including the difficult question of whether AChE and BChE resynthesis is indeed faster in young children than in adults, and at what developmental stage. They would also provide information on the potential importance of carboxylesterases and A-esterases at different ages. In the foreseeable future, however, we must continue to reason by analogy with rodent data and with the developmental profile of other liver drug metabolizing enzymes. In this context, it does appear reasonable to assume that the youngest infants will indeed be deficient in tissue carboxylesterase expression, and that expression of this enzyme will approach adult levels sometime in early childhood—probably in the 1-2 year bracket or sooner.

Two Panel members felt strongly that the studies presented by the Agency have limited application to understanding the effects of OP insecticides, specifically in children. While adverse effects related to mechanisms other than acetylcholinesterase inhibition are considered in the risk assessment for individual OP agents, there is concern that such possible effects could be "hidden" in the process of cumulative risk assessment. The evaluation of OP toxicity can be considered to belong in the realms of behavioral teratology and toxicology. James Wilson, who opened this field, delineated dose-response relationships of prenatal toxicants. At highest exposures, the outcome is fetal death; at somewhat lower doses, congenital defects; at lesser doses, growth retardation is seen; and finally, at the lowest exposures, functional deficits, most notably behavior, become visible. It is in this lowest exposure stratum that examination of OP toxicity should continue. The concern with the effects of OPs prenatally and postnatally is associated with the brain. This relates to the impact of OPs on children's function, and among their most critical functions is their ability to think, talk and pay attention. Not to include data on these outcomes excludes important variables in the assessment and therefore introduces important specification error. Wilson's work and the work of many others have shown that systematically measured behavior may demonstrate toxicological effects at lower doses than those that yield phenotypic or biochemical alterations.

These same Panel members further stated that EPA-listed studies of animal behavioral effects, some of which were not associated with cholinergic alterations, were conducted at doses of OP pesticides previously thought to be without effect. Levin and colleagues reported long term behavioral changes in offspring following maternal chlorpyrifos exposure. The nature of the changes (loss of sensitivity to cholinergic muscarinic antagonist) suggested that the behavioral effects were not cholinergic in origin. These and other data point to mechanisms besides AChE inhibition that may also be at work in OP toxicity. Thus, reliance on a single biochemical assay to measure brain damage may become problematic.

Expanding on this issue, the Panel members pointed out that when using a marker, in this case brain AChE levels as a marker for more proximate effects of OPs, one is required to calibrate it and determine its validity in estimating the process or event that it stands for. To determine this, it is necessary to measure both the marker and the process of interest (e.g., synaptogenesis, behavioral outcome) and determine the correlation between the two variates, the coefficient of determination, the sensitivity, specificity, and predictive power, both positive and negative, of the marker. These factors have precise meanings in science. Sensitivity is the probability that an outcome (e.g., impaired learning) will be identified by the marker. Specificity is the probability that the absence of such an outcome will be correctly identified. Predictive power positive is the probability that a positive test will identify a specified outcome. EPA has not indicated anywhere in its report that these important determinations have been accomplished. As a consequence, the amount of measurement error in the cumulative risk assessment is unknown. Since this measurement error is nonsystematic (neither systematically higher or lower AChE levels than the true values) and non-differential (not increased in subjects with higher brain AChE, etc., than with lower levels), the direction of the bias introduced by measurement error is toward the null. That is, it would tend to underestimate the size of the effect under study, in this case the sensitivity of children to OPs. From these points, these Panel members concluded that the EPA report contains substantial measurement and specification errors, and as a consequence, underestimates the risk of OPs for child health.

In general, however, it should be stressed that the cumulative risk assessment for the OP insecticides is indeed based on acetylcholinesterase inhibition and cholinergic toxicity. While non-cholinergic endpoints may weigh on the risk assessment of individual agents, the cumulative risk assessment is driven by cholinergic mechanisms initiated by acetylcholinesterase inhibition and related to consequent increases in acetylcholine, if the common mechanism for OP insecticides is acetylcholinesterase inhibition and cholinergic toxicity. Based on this endpoint, there is compelling evidence to support the conclusion of potentially higher sensitivity in infants and children.

Question 3.3

Please comment on the conclusions regarding the faster recovery in the young animal of AChE activity. Because there is no human information on the recovery of AChE in children compared to adults, please comment on the extent to which recovery of AChE in children should be factored into conclusions regarding potential risk to children.

The Panel agreed that given the conservation of neurodevelopmental processes across species, all aspects of this biological process identified to be critical in the rodent model should be taken into consideration when evaluating these compounds for their potential risk to children. The Panel raised some issues regarding the interpretation of the biological consequences of the apparent faster recovery of AChE activity in the young animals – that is the Panel had reservations about whether the faster recovery could be regarded as indicating a return to a completely normal state that is free of further neurodevelopmental consequences.

The Agency's background document provides information regarding what appears to be a faster recovery of AChE in young animals as compared to the adult. The available data are quite limited, however, and it is not possible to reach a conclusion regarding the dynamics of the underlying mechanisms of how this phenomenon occurs and its biological impact. Given the species conservation of many such biological processes, as well as the high degree of structural and functional homology between AChEs and ACh receptors in rats and humans, differential recovery rates should ultimately be factored into conclusions regarding possible risk to children. How this will be done in the absence of biological data is a question.

The general mechanism proposed for differential recovery rates deals with higher on-going macromolecular synthesis in immature tissues than adult tissue. There may also be differences in the ability of tissues to respond to AChE inhibition by inducing the synthesis of AChE. For example, some studies suggest that anticholinesterases can activate the transcription of AChE (Soreq and Seidman, 2001). These phenomena however, have not been adequately evaluated in animal models following OP exposure.

In order to fully appreciate the importance compensatory mechanisms in the younger animal, information is needed on relevant transmitter systems including synthesis rates, turnover rates, and equilibrium levels of the transmitters, as well as the pharmacology, numbers and binding capacities of the transmitter receptors. Finally, we need to know much more about the down stream effects of increased acetylcholine levels resulting from an inhibition of AChE.
Once this is known, we will have a better idea of exactly what the inhibition of AChE activity and its time to recovery may mean in the young animal.

The compensatory ability of the developing animal also shows itself in the relatively normal phenotypes seen with certain knockout animals and genetic mutants. One might take comfort in reasoning that adaptive mechanisms seen in experimental animal models are also likely to operate in humans. A strong caution needs to be raised, however, because compensatory and adaptive mechanisms can still lead to permanently abnormal outcomes.

Recovery of whole brain AChE does not necessarily imply return to a normal state, especially in the developing nervous system. That is because the formation of brain architecture and the elaboration and stabilization of synapses must continue during the period of neurochemical disruption. The possible result is a permanent alteration in the characteristics of synapses formed in the interval prior to, during, and following exposure. In addition, the replenishment of AChE may merely reflect synthesis of catalytically active but functionally deficient molecules, with regard to cholinergic neurotransmission.

As noted previously, however, when exposure periods are separated in time (4 day intervals between exposures), adult rats show more cumulative AChE inhibition and downregulation of receptors (Chakraborti et al., 1993). These findings suggest that the more robust recovery of AChE in immature animals indeed represents enhanced functional recovery. The major AChE expressed in nervous tissue is the so-called "synaptic" form (AChE-S). Chronic inhibition of AChE activity can lead to the expression of a unique transcript, referred to as the "read-through" form (AChE-R) that is secreted as a monomer (Grisaru, et al., 1999; Soreq and Seidman, 2001). This protein has the same enzyme kinetics as the synaptic form, and thus would appear in an enzyme assay as normal AChE. However, because the enzyme has a different distribution, it may not have the same functional impact as the normal AChE-S. The relevance of these findings to the issue currently under review remains to be determined, yet they raise concerns regarding the dynamics of the overall process of cholinesterase inhibition during development. With all of these biological processes, the consequences of such inhibition and replenishment would depend upon the stage of brain development occurring during this period.

ADDITIONAL COMMENTS

One Panel member provided comments on the exposure assessment for consideration in the selection of an appropriate FQPA uncertainty factor. This Panel member analyzed the EPA's treatment of exposures (e.g. dietary exposure). References in the document to 95th, 99th, 99.5th, 99.9th percentiles imply a view that such numbers bracket the high end exposures. A simple calculation of the corresponding consumption would show otherwise. This point can be illustrated by using the Agency's cumulative exposure for individuals 1-2 years old and assuming that the entire amount of exposure comes from a single chemical in a single food form of a commodity. For example, let's assume that the entire exposure is from azinphos methyl (AZM) in fresh apple or pear. The 1999 PDP single serving monitoring data showed that AZM was detected in 76.2% (1088 of 1427 samples) of apples at 0.01-0.55 ppm, and 43.2% (152 of 352 samples) of pears at 0.013-0.87 ppm. Taking into account the 0.1 of RPF for AZM, and using the highest detected residue (0.55 ppm for apple or 0.87 ppm for pears), the cumulative dietary exposure of 0.0002 mg/kg/day at the 95th percentile is equivalent to the consumption of either 1.3-1.9 oz. of apple or 0.8-1.2 oz. of pears. These levels of consumption do not appear to represent the high end of consumption even from just fresh apple or pears. Only as the cumulative exposure moves toward the higher distributional percentiles does it begin to appear more unlikely to be contributed from a single source.

This type of analysis is helpful to provide a context for exposure estimates in a cumulative risk assessment. Obviously, to choose an uncertainty factor to account for the exposure component we must know what percentile captures the reasonably expected high end. In this illustration, an argument can be made for an additional FQPA uncertainty factor if the benchmark for risk management decision is based on the 95th percentile of dietary exposure. Fortunately, for the exposure assessment, especially the dietary route, sufficient data are available for a much more informed decision. The Agency is encouraged to provide documentation that goes beyond the numerical exposure values and percentiles present in the Agency's background document.

REFERENCES

Bigbee, J.W., K.V. Sharma, E-L. Chan and O. Bogler. 2000. Evidence for the direct role of acetylcholinesterase in

neurite outgrowth in primary dorsal root ganglion neurons. Brain Res., 861:354-362.

Bigbee, J.W., K.V. Sharma, J.J. Gupta and J.L. Dupree. 1999. Morphogenic role for acetylcholinesterase in axonal outgrowth during neural development. Env., Health Perspect., 107(Suppl 1): 81-87.

Brimijoin, S. and C. Koenigsberger. 1999. Cholinesterases in neural development: new findings and toxicological implications. Environ. Health Perspect., 107(Suppl 1): 59-64.

Chakraborti, T.K., J.D. Farrar and C.N. Pope. (1993). Comparative neurochemical and neurobehavioral effects of repeated chlorpyrifos exposures in young and adult rats. Pharmacol. Biochem. Behav. 46:219-224.

Coronas, V., M. Durand, J.G. Chabot, F. Jourdan, R. Quirion. 2000. Acetylcholine induces neuritic outgrowth in rat primary olfactory bulb cultures. Neurosci., 98:213-219.

Drews, U. 1975. Cholinesterase in embryonic development. Prog. Histochem. Cytochem., 7:1-52.

Dupree, J.L and J.W. Bigbee. 1994. Retardation of neurite outgrowth and cytoskeletal changes accompany acetylcholinesterase inhibitor treatment in cultured rat dorsal root ganglion neurons. J. Neurosci. Res., 39:567-575.

Eskenazi, B.A., A.Boardman and R. Castorina. 1999. Exposure of children to organophosphate pesticides and their potential adverse health affects. Env. Health Perspective, 107, (suppl. 3) 409-419.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., and Goble, R. (2002) "Evaluation of Child/Adult Pharmacokinetic Differences from a Database derived from the Therapeutic Drug Literature," Toxicological Sciences, Vol. 66, pp. 185-200.

Grisaru, D., M. Sternfeld, A. Eldor, D. Glick and H. Soreq. 1999. Structural roles of acetylcholinesterase variants in biology and pathology. Eur. J. Biochem., 264:672-686.

Hattis, D., Ginsberg, G, Sonawane, B., Smolenski, S., Russ, A., Kozlak, M, and Goble, R. (2002, in press) "Differences in Pharmacokinetics Between Children and Adults—II. Children's Variability in Drug Elimination Half-Lives and in Some Parameters Needed for Physiologically-Based Pharmacokinetic Modeling," Risk Analysis.

Koenigsberger, C., S. Chiappa and S. Brimijoin. 1997. Neurite differentiation is modulated in neuroblastoma cells engineered for altered acetylcholinesterase expression. J. Neurochem., 69:1398-1397.

Layer, P.G. and E. Willbold. 1995. Novel functions of cholinesterases in development, physiology and disease. Progr. Histochem. Cytochem., 29:1-94.

Layer, P.G., T. Weikert and R. Alber. 1993. Cholinesterases regulate neurite growth of chick nerve cells in vitro by means of a non-enzymatic mechanism. Cell Tiss. Res., 273:219-226.

Mesulam, M.M., A. Guillozat, P. Shaw, A. Levey, E.G. Duysen and O. Lockridge. 2002. Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. Neurosci., 110:627-639.

Pope, C.N. and Liu, J. (1997). Age-related differences in sensitivity to organophosphorus pesticides. Environ. Toxicol. Pharmacol. 4:309-314.

Sharma, K.V. and J.W. Bigbee. 1998. Acetylcholinesterase antibody treatment results in neurite detachment and reduced outgrowth from cultured neurons: further evidence for a cell adhesive role for neuronal AChE. J. Neurosci. Res., 53:454-461.

Soreq, H. and S. Seidman. 2001. Acetylcholinesterase – New roles for an old actor. Nature Neuroscience, 2:8-16.

Sternfeld, M., G-L. Ming, H-J. Song, K. Sela, R. Timberg, M-M. Poo and H. Soreq.1998. Acetylcholinesterase enhances neurite growth and synapse development through alternative contributions of its hydrolytic capacity, core protein and variable C-termini. J. Neurosci., 18:1240-1249.

Wessler, I., C.J. Kirkpatrick and K. Racke. 1998. Non-neuronal acetylcholine, a locally acting molecule, widely distributed in biological systems: Expression and function in humans. Pharmacol. Ther., 77:59-79.

Xie, W., J.A. Stribley, A. Chatonnet, P. J. Wilder, A. Rizziono, R.D. McComb, P. Taylor, S.H. Hinrichs and O. Lockridge. 2000. Postnatal developmental delay and supersensitivity to organophosphate in gene-targeted mice lacking acetylcholinesterase. J. Pharm. Exp. Therap., 293:896-902.

Zheng, Q., Won, Y., Olivier, K. and Pope, C. (2000). Comparative cholinergic neurotoxicity of oral chlorpyrifos exposures in preweanling and adult rats. Toxicological Sci. 55:124-132.