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

OFFICE OF
PREVENTION, PESTICIDES AND TOXIC
SUBSTANCES

December 17, 2008

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held September 16-18, 2008 on the Agency's Evaluation of the Toxicity Profile of Chlorpyrifos

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

FROM: Sharlene R. Matten, Ph.D.
Designated Federal Official
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Steven Knott, Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Frank Sanders for
Director
Office of Science Coordination and Policy

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, VA on September 16-18, 2008. This report addresses a set of scientific issues regarding the Agency's Evaluation of the Toxicity Profile of Chlorpyrifos.

Attachments

cc:
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James J. Jones
Anne Lindsay
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Margie Fehrenbach
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William Diamond
Joan Harrigan-Farrelly
Tina Levine
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SAP Minutes No. 2008-04

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

The Agency's Evaluation of the Toxicity Profile of Chlorpyrifos

September 16-18, 2008
FIFRA Scientific Advisory Panel Meeting
held at the
Holiday Inn - Rosslyn
Arlington, Virginia

NOTICE

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). The meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of the meeting minutes does not represent information approved or disseminated by the Agency. The meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of these meeting minutes do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the Environmental Protection Agency, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. FQPA Science Review Board members serve the FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Sharlene R. Matten, Ph.D., SAP Designated Federal Official, via e-mail at matten.sharlene@epa.gov.

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

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FIFRA Scientific Advisory Panel Meeting
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Kenneth M. Portier, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel

Date: 12/17/08

Sharlene R. Matten, Ph.D.
Designated Federal Official
FIFRA Scientific Advisory Panel Staff
Date: 12/17/08

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
September 16-18, 2008

The Agency's Evaluation of the Toxicity Profile of Chlorpyrifos

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed its review of **The Agency's Evaluation of the Toxicity Profile of Chlorpyrifos.** Advance notice of the SAP meeting was published in the *Federal Register* on **July 9, 2008 and August 28, 2008**. The review was conducted in an open panel meeting September 16-18, 2008 held at the Holiday Inn-Rosslyn, Arlington, Virginia. Dr. Kenneth M. Portier chaired the meeting. Dr. Sharlene R. Matten served as the Designated Federal Official. Dr. Tina Levine, Director, Health Effects Division, Office of Pesticide Programs (OPP), provided opening remarks at the meeting. Presentations of technical background materials were provided by Dr. Anna Lowit and Ms. Deborah Smegal, MPH, Health Effects Division, OPP and by Dr. Ginger Moser, EPA-ORD-National Health and Environmental Effects Research Laboratory (NHEERL). Additional technical assistance was provided by Dr. John Liccione and Dr. John Doherty of the Health Effects Division, OPP.

Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro -2-pyridinyl phosphorothioate) is a broad-spectrum, chlorinated organophosphorus (OP) insecticide. Chlorpyrifos is one of the most widely used OPs in the U.S. In 2000, nearly all residential uses were voluntarily cancelled by Dow AgroSciences, LLC. However, chlorpyrifos continues to be used extensively in commercial agriculture. Since 2000, there has been extensive research on various aspects of chlorpyrifos toxicity, particularly on effects in animals and humans from gestational and postnatal exposure. Many new studies in rats investigating different endpoints including acetylcholinesterase (AChE) inhibition and adverse effects on the developing brain are now available. In addition, manuscripts have recently been published from three cohorts of pregnant women and children exposed *in utero* to organophosphates (OPs). At this time, the Agency is re-evaluating the extent to which toxicity endpoints and extrapolation/uncertainty factors for chlorpyrifos require updating based on this new information. The Agency's issue paper and associated appendices contain the proposed updates and the scientific foundation for the proposed revisions. The contents and conclusions drawn in the issue paper and appendices are preliminary. The ultimate goal of the Agency's on-going work is to improve the scientific support for the Agency's risk assessment. This will be accomplished by 1) evaluating new data on potentially susceptible subpopulations and 2) incorporating improved approaches, e.g., benchmark dose modeling instead of relying on no-observed-adverse-effect levels (NOAELs) for points of departure; and using extrapolation factors based on data instead of relying on default factors to account for differences in animals and humans and among humans. The Agency has progressed to a point in the review that feedback from the FIFRA SAP would be helpful.

PUBLIC COMMENTERS

Oral statements were presented by:

1) Daland Juberg, Ph.D., Dow AgroSciences, LLC
2) Charles Timchalk, Ph.D., DABT, Battelle Center for Biological Monitoring and Modeling
3) Carol Burns, Ph.D., Dow Chemical Company
4) Pamela Mink, Ph.D., MPH, Department of Epidemiology, Emory University
5) Michael Bartels, Ph.D., Dow Chemical Company
6) Douglas Weed, M.D., MPH, Ph.D., DLW Consulting Services
7) Robert Sielken, Ph.D., Sielken & Associates Consulting, Inc.
8) Michael Dourson, Ph.D., DABT, ATS, Toxicology Excellence for Risk Assessment (TERA)
9) Mr. Ray McAllister, Crop Life America
10) Elliot Gordon, Ph.D., Elliot Gordon Consulting, LLC
11) Jennifer Sass, Ph.D., Natural Resources Defense Council (NRDC) and on behalf of Pesticide Action Network North America (PANNA)
12) Michael Fry, Ph.D., American Bird Conservancy
13) Robin M. Whyatt, Ph.D., and Virginia A. Rauh, Ph.D., Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University

Written statements were provided by:

1) Torka S. Poet, Ph.D. and Charles Timchalk, Ph.D., DABT, Battelle Center for Biological Monitoring and Modeling, Battelle
2) Charles Timchalk, Ph.D., Battelle Center for Biological Monitoring and Modeling
3) David Eaton, Ph.D., DABT, FATS, Center for Ecogenetics and Environmental Health and Associate Vice Provost for Research, University of Washington on behalf of the authors of Eaton et al. 2008
4) Theodore Slotkin, Ph.D., Professor of Pharmacology and Cancer Biology and Psychiatry and Behavioral Sciences and Neurobiology and Director of Graduate Studies, Integrated Toxicology and Environmental Health Program, Duke University Medical Center
5) Scott Phillips, M.D., Department of Medicine, University of Colorado Health Sciences Center
6) Pamela Mink, Ph.D., MPH, Department of Epidemiology, Emory University
7) Douglas Weed, M.D., MPH, Ph.D., Founder and Managing Member, DLW Consulting Services, LLC
8) Kenneth D. Racke, Ph.D., Dow AgroSciences, LLC
9) Gary J. Mihlan, Ph.D., CIH and Walter Schmitt, Ph.D., Bayer CropScience
10) Michael Dourson, Ph.D., DABT, ATS, Bernard Gadagbui, Ph.D., DABT, and Lynne Haber, Ph.D., DABT, Toxicology Excellence for Risk Assessment (TERA)

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

The Panel acknowledged the extent of the chlorpyrifos database and commended the Agency for preparing a comprehensive document considering the scientific evidence as a whole. Some of the more significant new data available to the Agency since the preparation of the 2000 chlorpyrifos risk assessment comes from three large prospective cohort studies of pregnant women and their children. Throughout the two-day discussion, Panel members referred to the results of these studies in an attempt to integrate their findings with the toxicology data from laboratory animal experiments. Despite the large volume of data available to address the risks of chlorpyrifos exposure from agricultural uses, several Panel members were concerned that a high degree of uncertainty is evident in the available data, particularly under low dose, chronic conditions. Uncertainty was expressed in attributing observed adverse effects to chlorpyrifos in the epidemiological studies where exposure was to metabolites of chlorpyrifos or to a mixture of chlorpyrifos and two additional anticholinesterase insecticides. The Panel agreed that the epidemiological studies have utility for risk assessment, but not as the principal basis for characterization of the point of departure (PoD). There was extensive discussion on experimental and epidemiological studies, e.g., of low-dose exposure in animals, of exposures in agricultural and pesticide handlers, and of the mode of action including the need to develop chlorpyrifos-specific PBPK models, that could provide the information needed to address critical data gaps and reduce uncertainty.

1. Metabolism & Toxicokinetics

The Panel concluded that a weight of evidence evaluation of available information supports the Agency's conclusions that 1) sensitivity to the adverse effects of chlorpyrifos is influenced by age, with young animals having lesser total ability to detoxify chlorpyrifos and many other organophosphorus compounds, and 2) that the age-dependent sensitivity observed in experimental animals is due mostly to toxicokinetic (TK) differences between juveniles and adults instead of only being the result of toxicodynamic (TD) differences.

The Panel agreed that current scientific data on chlorpyrifos uptake, transport, sequestration and excretion suggest that individual differences in metabolism and transformation will explain much of the variability seen in these factors, but that other potential TK differences between juveniles and adults should not be dismissed. For instance, the high respiratory rate of children may enhance the absorption of chlorpyrifos present in the air. Children have a greater cardiac output compared to adults, together with their less developed blood-brain barrier; facilitate some of the chlorpyrifos-oxon reaching the brains of exposed infants.

Some panel members questioned whether detoxication of the oxon plays a significant role in explaining the differential susceptibility between adults and juveniles at lower levels of chlorpyrifos exposure. Toxicokinetic differences are less likely to be relevant under low dose conditions compared to high dose conditions where enzyme systems may become saturated. Human tissue data specific to enzyme-mediated detoxication are minimal, however. While blood levels of detoxifying enzymes (e.g., PON1, carboxylesterases) have been studied, data on chlorpyrifos detoxication in specific organs (e.g., the liver) from humans are limited, making it

difficult to draw firm conclusions about the role of activation and detoxication in explaining differences in susceptibility to chlorpyrifos toxicity between human infants, children, and adults.

The Panel also agreed with the Agency's conclusion that "pregnant animals and humans may be somewhat more sensitive to chlorpyrifos than non-pregnant adults" based on the expected role of key detoxication enzymes (e.g., paraoxonase, P450 isozymes) in modulating levels of chlorpyrifos in animal studies. However, the Panel noted that there are relatively small differences in levels of these key enzymes between pregnant and non-pregnant animals. At dose levels expected from environmental exposures the importance of such small differences in enzyme levels is unknown, but the data suggest a reduced capacity to detoxify chlorpyrifos during pregnancy.

2. Cholinesterase Inhibition

Panel members agreed with the Agency's conclusion that postnatal rats are more sensitive than adult rats to increased cholinesterase inhibition and lethality following acute exposures. Increased susceptibility appears to be the result of the juvenile animals' lower capacity to detoxify either chlorpyrifos or the chlorpyrifos-oxon. The Panel was uncertain about how to interpret the data describing differences in cholinesterase inhibition between post-natal animals and adults following repeated chlorpyrifos dosing. The evidence suggests that younger animals are less sensitive than adults to cholinesterase inhibition from low dose repeated exposures, likely due to a lack of enzymes capable of converting chlorpyrifos to the oxon form in younger animals. In essence, the total acetylcholinesterase activity in the younger animal's tissues increases more effectively in between exposures, leading to lesser accumulation of inhibition over time than in adults. However, the role of the activation and detoxication pathways in producing differential cholinesterase inhibition and cholinergic effects at low dose repeated exposures is not clearly defined by the available data.

The Panel agreed with the Agency's preliminary conclusion that data showing less cholinesterase inhibition after repeated dosing during gestation may not reflect the true potential toxicity to the fetus. The data indicate, however, that the level of cholinesterase inhibition depends on the time of sampling following exposure. Developing rats would be expected to show less cholinesterase inhibition than would adults, with increasing length of time of measurements vs. dosing. Two mechanisms may explain this phenomenon: 1) a higher rate of recovery of the inhibited cholinesterase molecules and/or 2) an increase in cholinesterase enzyme synthesis, in the developing rat. There is little experimental evidence to indicate differences in enzyme reactivation following inhibition in cholinesterases from either young or adult tissues. The difference in rate of synthesis of new enzyme molecules is likely the dominant factor. A more accurate comparison of cholinesterase inhibition between the developing animal and the adult could be determined if sample collection coincided with the time of the peak cholinergic effect.

3. Laboratory Studies on the Developing Brain

In general, the Panel agreed with the Agency's conclusion that gestational or early postnatal exposures can lead to neurochemical and behavioral alterations that persist into adulthood. A number of rodent studies suggest that prenatal or postnatal exposures may lead to long-term

neurobehavioral changes in motor and cognitive behaviors. The Panel questioned the meaning of the phrases "at sufficiently high exposures" and "persisting into adulthood after any initial AChE inhibition has reversed." Some panelists stated that a "sufficiently high exposure" should be defined on the basis of a dose that induces brain cholinesterase inhibition. They noted that the majority of experimental animal studies reviewed by the Agency used doses of about 1 mg/kg. This dose (exposure level) should be sufficient to inhibit brain cholinesterase activity and at the same time causes neurobehavioral effects in most age groups.

Some members questioned the experimental methods used in some of the animal studies as well as the interpretation and application of the results of neurobehavioral testing in animals for risk assessment. Several Panel members felt that while behavioral findings are consistently reported in the literature, the types of behavioral activities reported as significant were not always the same. It was acknowledged that the study outcomes could be affected by 1) the route of administration of chlorpyrifos, 2) the developmental period of exposure, 3) the methods used to measure changes in behavioral domains, and 4) the choice of dependent variables. Panel members agreed with the Agency's expressed caution on the use of dimethyl sulfoxide (DMSO) as a vehicle because of its intrinsic toxicity and potential influence on absorption. In addition, uncertainly was expressed about potential interactions between DMSO and low doses of chlorpyrifos and the effect of this interaction on the developing animal.

The Panel agreed with the Agency's determination that insufficient data exist to clearly identify a specific mode of action for effects in the developing nervous system for use in risk assessment. Some panel members believed that studies cited by Eaton et al. (2008) were indicative of possible non-cholinergic modes of action. Other panel members disagreed with this conclusion. They stated that the effects on blood cholinesterase are considered protective for the observed effects (i.e., occurring at lower doses); however, nothing is implied about mode of action, except in the context of species and interindividual variability in toxicokinetics. Most of the Panel stated that the available information does not allow behavior to be considered as a point of departure. These panelists stated that cholinesterase inhibition should continue to be used for PoD until, at such time; an alternative mode of action is identified and validated. Collaboration between EPA and developmental neurotoxicologists would help identify enzyme inhibition occurring in discrete brain sites at critical periods of development.

4. Epidemiology Studies in Children and Mothers

The Panel agreed with the Agency's conclusions regarding the potential utility of the epidemiological data from the three cohort studies (Columbia University in New York, Mt. Sinai Hospital in New York, and the University of California at Berkeley) in broadly characterizing risk of chlorpyrifos exposure to pregnant women, fetuses, infants, and children. They also concurred that these studies should not be considered as the principal basis for characterization of the PoD. The Columbia study was considered the most epidemiologically-sound and to have adequately addressed selection and information biases to the best extent possible. It was also considered the most robust and appropriate in assessing chlorpyrifos developmental toxicity because specific measurements of exposure to chlorpyrifos in neonates and children (i.e., the study populations) were obtained. Confounding factors in the Mt. Sinai and Berkeley studies, particularly the fact that exposures were based on OP and carbamate metabolites and that

chlorpyrifos was not specifically measured, reduce their utility in a quantitative context for risk assessment. Several panel members pointed out that although the Agency classified the study population's exposure as "high" for a residential setting, it would be considered a "low" exposure for a population of agricultural workers or pesticide handlers.

The weaknesses inherent to observational studies were discussed. Given the paucity of human epidemiological studies of OP insecticides in children, the Mt. Sinai and Berkeley studies do provide useful information for risk assessment. For example, these studies provide data demonstrating abnormal reflexes in neonates that is not available from the Columbia cohort study. In addition, the Mt. Sinai cohort study considered paraoxonase activity as a factor in the analyses that may have relevance for human toxicity. There is uncertainty in the exposure estimates in the Columbia study because measurements were derived from a single time point (maternal and/or cord blood). Notwithstanding this uncertainty, the Panel felt that the results from the Columbia study raise concerns for adverse neurodevelopmental effects in children exposed *in utero* to environmental levels of chlorpyrifos although there was confounding by the presence of other anticholinesterase insecticides. The majority of issues raised by the Panel were points of clarification or issues that if addressed could strengthen the analysis and provide the Agency with better and more useful data for risk assessment.

Overall, the Agency's conclusion that chlorpyrifos could have contributed to the birth and neurodevelopmental outcomes noted in the three cohort studies was supported. The Panel agreed with the Agency that: a) exposures to multiple cholinesterase-inhibiting insecticides in combination cannot be ruled out as contributing to the birth and neurodevelopmental outcomes; b) a potential synergistic and/or additive effect of these compounds does not rule out a role for chlorpyrifos in contributing to the adverse outcomes; and c) it cannot be determined if chlorpyrifos is the sole contributor to the observed outcomes. These conclusions are supported by the Columbia study finding of a crude dose-response relationship to chlorpyrifos levels which persisted even after controlling for exposure to other pesticides.

When the results of the three cohort studies (with an emphasis on the Columbia study) are considered along with the findings from experimental studies in animals, the Panel concluded that maternal chlorpyrifos exposure would likely be associated with adverse neurodevelopmental outcomes in humans. Some panelists indicated that if the associations between chlorpyrifos exposure and the reported outcomes are causally related, then it is possible to conclude that the mechanism of action might be independent of cholinesterase inhibition. Exposures to multiple cholinesterase-inhibiting pesticides or other neurotoxicants may result in additive or interactive effects. This information might be useful when considering cumulative exposures to these pesticides.

5. Human Information Available for Risk Assessment

The Panel generally agreed with the Agency's conclusion that due to their limitations, the epidemiological data currently available are useful primarily for hazard identification. The Panel disagreed on whether the current epidemiological data provide sufficient evidence to suggest that the uncertainty factor for the cholinesterase inhibition endpoint be changed to accommodate the possibility of neurodevelopmental effects from low-level *in utero* exposures of chlorpyrifos. The

majority of the Panel agreed that the current epidemiological data do not provide sufficient evidence to increase the uncertainty factor for the cholinesterase inhibition to accommodate the possibility of neurodevelopmental effects. However, some panel members felt strongly that the current epidemiological data do provide evidence to indicate that the margin of safety should be increased. The Panel recommended that the Agency conduct a full formal weight of evidence evaluation for causality of the reported associations between exposure to chlorpyrifos and neurodevelopmental outcomes in the existing epidemiological database.

The Panel recommended that the Agency continue its collaboration with Columbia University researchers in analyzing the epidemiological data. The Agency is encouraged to continue open discussions with the study researchers (and the Centers for Disease Control and Prevention, as appropriate) to seek clarification on the level of confidence in the reported exposure levels. The Agency should then attempt to use the cohort data quantitatively to inform the risk assessment process, such as in a boundary setting exercise.

The use of physiologically-based pharmacokinetic (PBPK) modeling would enable estimation of exposure dose metric for multiple sources of exposure, e.g., air, food, water. The Panel also agreed with the concept of using a PBPK model to examine individual intra-species variations as well as inter-species differences. While the potential contribution of the chlorpyrifos PBPK model being developed was recognized, some panelists believed that the Agency should also pursue a simpler PBPK model specifically applicable to the chlorpyrifos data that would be available in a relatively short timeframe. The Panel concluded that the epidemiological data may be used for bounding exposure levels, and in conjunction with PBPK models, address current or potential human exposures and to determine the final reference dose or reference concentrations.

The Panel agreed with the Agency's approach to deriving a dermal absorption factor using data from the deliberate dosing in human studies. This approach is especially valuable if no other sources of data are available. However, the current 3% absorption factor should be further refined by considering different exposure scenarios and adjusting for expected underestimation of exposure. The Panel also agreed with the Agency's scientific analysis that these deliberate dosing studies cannot be used to directly establish a PoD or UFs, but indicated that these data might be used as bounding levels similar to what was suggested by the Panel concerning the data from the epidemiological studies.

6. Points of Departure (PoD) for Risk Assessment

The Panel was presented with three options for deriving points of departure for acute and chronic chlorpyrifos exposure from animal studies for extrapolating human risk. Panel members expressed difficulty in separating the discussion of points of departure from that of uncertainty factors with the result that the discussion of these two issues overlapped in many areas.

The Panel agreed that Option 3 was the most favorable option for deriving points of departure for acute and chronic exposure. There was also general agreement among Panel members with the Agency's proposal to use the dermal and inhalation studies from their 2000 assessment as a basis to develop points of departure for these exposure routes, taking into account sensitive life stages and adjusting for dosing regimen. Option 3 was chosen principally on the basis that the proposed point of departure for acute exposure takes into account all life stages, is based on

benchmark doses (an advantage over the NOAEL or LOAEL), and represents the results of several studies.

Based on available data, most of the Panel members (a few members disagreed) stated that the PoD presented in Option 3 is also believed to be protective for effects on the developing brain, although it is based on cholinergic effects. However, this conclusion has associated uncertainties; the lack of information on the mode of action in inducing the observed behavioral effects and evidence from *in vivo* and *in vitro* studies that show non-cholinergic modes of action are likely to be involved in the adverse developmental neurotoxicity and behavior endpoints. The Panel encourages EPA to explicitly address these uncertainties when deriving the reference dose/concentrations.

The effects on neurobehavioral development observed in the three epidemiological studies, the uncertainties surrounding the mode(s) of action following longer term exposure, and the need to account for potential cumulative effects support the lower point of departure for repeated exposures used by the Agency in its 2000 risk assessment. The Panel also recommended that the Agency investigate the possibility of conducting a benchmark dose assessment for the chronic point of departure determination.

The Panel recommended that the Agency consider development of an appropriate study using doses spanning and below those expected to produce cholinergic effects with the most sensitive administration method and the most sensitive life stage. Such a study would examine developmental neurotoxicity and behavior outcomes along with cholinesterase inhibition. This would likely help elucidate non-cholinergic modes of action for developmental neurotoxicity and behavior effects.

7. Extrapolation/Uncertainty Factors

After review of available data, the Panel concurred with the Agency that paraoxonase-1 (PON1) status cannot be ruled out as a determinant of chlorpyrifos toxicity, particularly for the fetus and the young child. Actual human exposures vary between bystanders and applicators (and everyone in between) and it would be difficult to define what constitutes a low level of exposure in humans. The Panel disagreed on whether the PON1 data alone could be used to address uncertainty. The majority of panel indicated that these data should not be used out of context until rate limiting step(s) is/are identified. The use of the PON1 data without such information was considered by some to be a misinterpretation or misuse of the IPCS guidance for determining chemical-specific adjustment factors (CSAFs).

Some of the Panel indicated that chlorpyrifos may produce effects in the fetus that would be manifest later at the juvenile stage or later on in life (e.g. adulthood). They stated that adverse neurodevelopmental effects in the fetus, neonates, and young children should be considered the most important endpoints for assessing chlorpyrifos toxicity. The Panel noted that there does seem to be a different susceptibility between fetuses and neonates compared to adults. Because specific modes of action have yet to be identified for these effects, available data are inadequate to inform inter-species and intra-species differences in TD and TK. Therefore, the application of default uncertainty factors for TD and TK considerations was recommended by the Panel.

The Panel noted the uncertainties surrounding the role of PON1 and its genetic polymorphisms in humans exposed to chlorpyrifos and other cholinesterase inhibitors. Some panel members emphasized the lack of data in animals and humans on the involvement of PON1 in detoxication pathways, especially in situations of low dose exposures. Most of the Panel encouraged the Agency to use PBPK modeling to assess the overall impact of PON1 on toxicity over a range of chlorpyrifos exposures. While other panel members recommended that the Agency gather data (both *in vivo* and *in vitro* data) on animal and human enzyme kinetics and analyze carefully the effects as related to the PON-192 Q/R polymorphism. The information on the overall contribution of PON1 Q192R polymorphism on the deactivating pathways and potentially rate-limitanting components will be very useful when examined in light of future PBPK models.

The Agency proposed two options for deriving an intra-species TK (human variation) uncertainty factor: 1) a 12-fold data-derived factor based on the PON1 data or 2) the default three-fold factor. The majority of the Panel supported the use of the default three-fold factor. These panel members disagreed with the Agency's approach to use PON1 genetic polymorphisms to derive intra-species uncertainty factor. They stressed that PON1 is only one downstream enzyme in a complex metabolic pathway and that the PON1 genotype alone is insufficient to predict human variability. One panel member added that if chlorpyrifos acts directly on certain brain targets to elicit developmental neurotoxicity without the need of activation to the chlorpyrifos oxon (the substrate for PON1), then the role of PON1 for neurodevelopmental toxicity would be irrelevant. On the other hand, several panelists stated that the available PON1 data support an uncertainty factor of 12 for the intra-species factor to account for potential developmental neurotoxicity of chlorpyrifos as shown in the epidemiological studies. These members preferred the UF_{HK} of 12-fold, rather than the default of 3-fold, because these were the only two choices proposed. However, none of the panel members endorsed the CSAF approach used by the Agency to identify the factor of "12-fold" calculated based on chlorpyrifos-oxonase and encouraged the Agency to pursue other approaches based on the mode of action (i.e., 2005 IPCS CSAF guidelines).

The Panel did not reach consensus on one specific uncertainty factor for inter-species TK differences. Most of the panel concurred with the Agency's proposal to stay with the default three-fold factor for inter-species TK differences and add no additional uncertainty factor for developmental and behavior neurotoxicity. These panel members noted that the most sensitive effect appears to be AChE inhibition with a $BMDL_{10}$ (i.e., lower confidence limit on the benchmark dose calculated to a 10 percent effect level) for the red blood cell (RBC) AChE inhibition after repeated exposure at 0.03 mg/kg/day; whereas, the lowest dose tested in the developmental neurotoxicity studies in dams (0.3 mg/kg/day) was 10-fold greater and failed to produce observable behavioral effects in the offspring. On the other hand, a few panel members disagreed with this assessment and recommended that the Agency should apply a default uncertainty factor of 100 to the points of departure based on the cholinesterase inhibition endpoint and further consider the use of an additional uncertainty factor to address the concerns for developmental and behavioral neurotoxicity as observed in both the animal and epidemiological studies.

The Panel generally favored the use of a PBPK model to integrate key TK and TD factors and evaluate their contributions to the endpoints of interest (e.g., cholinesterase inhibition) for various chlorpyrifos exposure scenarios and for various life stages. In addition, the dose response relationships used in these models need to reflect the understanding gained from animal studies, what is known about inter-species and intra-species differences, and be validated against data from deliberate dosing studies in humans and epidemiological studies. The Panel encouraged the Agency to continue examining the importance of all enzymes in the metabolic pathway for chlorpyrifos, and acknowledged the data gaps for carboxylesterases and P450 enzymes.

The Panel discussed alternative approaches for calculating the uncertainty factors in the chlorpyrifos risk assessment.

DETAILED RESPONSES TO CHARGE QUESTIONS

1. Metabolism & Toxicokinetics (Issue Paper Section 3.1, Appendix A):

The Agency has performed a literature review of *in vivo* and *in vitro* studies on the metabolic profile and toxicokinetic (TK) properties of chlorpyrifos with particular focus on age-dependent and lifestage sensitivity.

a. The Agency has concluded that age-dependant sensitivity, at least in part, is derived based on toxicokinetic (TK) differences between juveniles and adults. These TK differences lead to reduced ability to detoxify chlorpyrifos or the oxon in juvenile animals. *Please comment on the Agency's conclusion and the scientific support for or against this conclusion.*

Panel Response

The Panel supported the Agency's conclusion. A weight of evidence evaluation of the available information shows that sensitivity to the adverse effects of chlorpyrifos is influenced by age, with young animals having lesser total ability to detoxify chlorpyrifos as well as many other OPs. The Panel agreed with the Agency's conclusion that the age-dependent sensitivity is mostly based on TK differences between juveniles and adults rather than soley on TD differences.

Chlorpyrifos is activated by oxidative desulfuration to the oxon which is further detoxified by cytochrome P450-mediated dearylation. A second detoxication mechanism involves A-esterases (PON1 activity towards chlorpyrifos-oxon) and B-esterases (carboxylesterases, cholinesterases) operating only on oxon that is available to them following the bioactivation reaction, and hence these are the second enzymes in the pathway. These reactions take place mainly in the liver, although they can also occur to a minor extent in other tissues.

The Panel agreed that, based on the current scientific data, differences in metabolism and biotransformation seem to play a more prominent role than age-dependent differences in the uptake, transport, sequestration, and/or excretion of chlorpyrifos. They also indicated that other potential TK differences between juveniles and adults should not be dismissed. For instance, it

was mentioned that the high respiratory rate of children enhances their absorption of any chlorpyrifos present in the air. Children have a higher skin surface-to-weight ratio that favors dermal absorption and a higher permeability of the small intestine epithelium that facilitates absorption by the oral route. The relatively greater cardiac output of juveniles, together with their less developed blood-brain barrier may facilitate some of the circulating chlorpyrifos-oxon eventually reaching the brain. Children have a greater cardiac output compared to adults, facilitating some of the chlorpyrifos-oxon reaching the brain. The blood-brain barrier in humans is not complete until 6 months of age (Rodier, 2004), facilitating some of the circulating chlorpyrifos-oxon in reaching the brains of exposed infants

Polymorphisms in several of the enzymes involved in chlorpyrifos metabolism can lead to variability in an individual's ability to detoxify the chlorpyrifos-oxon. Some enzymes are also inducible which may also contribute to variability in response to chlorpyrifos (and its metabolites). Most of the studies evaluating biotransformation capacity with acute sensitivity are correlative in nature, however, and are not mechanistic. While isoform expression of cytochrome P450 (CYP450) can be markedly different between juvenile and adult animals, the relative roles these isoforms play in sensitivity to chlorpyrifos are not clear. Juveniles appear to be less efficient than adults at activating chlorpyrifos to its oxon metabolite using CYP450. Nevertheless, the dearylation-to-desulfuration ratio in adults and juveniles is unknown, but may differ as a function of age.

Choi et al. (2006) stated that the adult (human) liver plays an important role in detoxication of chlorpyrifos and that in the liver the oxon does not accumulate to be released into the bloodstream. Hunter et al. (1999), however, suggested that the fetal liver does not play a significant role in detoxication. Some studies suggest that chlorpyrifos oxon formed in the liver does not escape to enter the bloodstream (Sultatos et al., 1984). Poet et al. (2003) stated that at low oral doses of chlorpyrifos, CYP/PON1 in the intestine and liver may effectively remove the oxon from circulation prior to systemic exposures. By contrast, individuals with lower levels and/or activity of the detoxication enzymes may have these pathways become saturated at high exposure levels. At high acute dosages, as is frequently the case in laboratory animal experiments, the detoxication systems are operating at maximal levels and the differences between adults and juveniles are more readily apparent. However these saturating conditions are less likely to occur at environmental exposure levels. If the detoxication mechanisms are not saturated, then their effectiveness in adults and juveniles may not be as different as experimental animal data with high exposures suggest. The lack of available data on maturational differences in tissue (non-blood) detoxication capacity by A-esterases and other pathways limits extrapolation of relative sensitivity in infants based on differential detoxication.

Timchalk et al. (2006) reported that the rate of detoxication (measured as the formation of the chlorpyrifos specific metabolite, 3,4,6-trichloro-2-pyridinol, referred to as TCP) exceeded the rate of activation of chlorpyrifos (measured as chlorpyrifos oxon formation) in rats across age groups from 5 days to adulthood, with exposures ranging from 1-10 mg/kg. This would suggest that at similar exposures, inactivation processes may outweigh the net activation. At higher exposures, inactivation might become compromised. At low chlorpyrifos concentrations, the formation of non-toxic metabolites is highly favored in the fetus (Buratti et al., 2006).

In evaluating the role of toxicokinetics and more specifically the lower detoxication potential in juveniles compared to adults, the Panel proposed that the relatively inefficient bioactivation reaction, especially in juveniles, would not likely lead to high concentrations of the oxon at environmental exposure levels. The detoxication enzymes would not be at saturating substrate concentrations for efficient inactivation. Age differences in toxicokinetics are likely to be important at high dosage levels, but less likely to be relevant at lower dosages where enzymes are not saturated.

The two detoxication pathways studied the most, in relation to age-related differences in sensitivity, are carboxylesterases and A-esterases (primarily paraoxonase-1 referred to as PON1). Specific activities of liver carboxylesterases (CarbE) increased with age in male rats (Atterberry et al., 1997) which indicated the presence of more protective esterases in the adult as compared to the juvenile animal. The lower carboxylesterases activity in blood and tissues in juveniles appears to play a role in the differential sensitivity of juvenile animals to chlorpyrifos compared to adults (Morgan et al., 1994; Moser et al., 1998; Karanth and Pope, 2000). However, humans do not have carboxylesterases in the plasma (Li et al., 2005) and some data suggest relatively minimal differences in liver carboxylesterases between infants and adults (Pope et al., 2005). On the other hand, juveniles contain low serum albumin levels as compared to adults, and albumin has been reported to hydrolyze chlorpyrifos-oxon (Sogorb et al., 2008), and is capable of protecting AChE (acetyl cholinesterase) "in vitro" from inhibition at low concentrations of this toxic metabolite (~ 0.1 μM).

Although BChE -/- (butyrylcholinesterase knockout) mice showed relatively similar toxic responses as BChE +/+ mice following chlorpyrifos oxon exposure (Duysen et al. 2007), the contribution of BChE to the metabolism of chlorpyrifos-oxon in humans remains to be determined. This finding can be explained by the high carboxylesterase activity in mouse plasma. Given that carboxylesterase is lacking in human plasma, the protective role of BChE cannot be disregarded.

The Panel was not aware of data specifically demonstrating that chlorpyrifos can be bioactivated in the brain, the main target organ of this compound. However, there are data from "*in situ*" experiments in rats that show bioactivation of parathion (another insecticide of the organophosphorothioate class) in the brain in the intact organism (Chambers et al., 1989 and 1991). Buratti et al. (2005) hypothesized that independent of the chemical structures, organophosphorothioates are bioactivated by the same CYP450s. Since chlorpyrifos is bioactivated less efficiently than parathion by rat liver microsomes (Ma and Chambers, 1994, 1995), it is certainly possible that bioactivation of chlorpyrifos occurs in the brain since very low desulfuration activity of various phosphorothioate insecticides, including chlorpyrifos, has been reported in both microsomal and crude mitochondrial fractions from brain (Chambers and Chambers, 1989). The brain desulfuration activities of these phosphorothioates generally correlate well with the toxicity and may be important in determining their overall acute toxicity levels (Chambers and Chambers, 1989). Thus, extrahepatic sites of activation likely play an important role in mediating the acute toxicity of chlorpyrifos (Sultatos et al., 1984).

PON1 is differentially expressed throughout maturation with lower levels in younger animals being associated with higher acute sensitivity (Mortensen et al., 1996; Li et al., 1997; Karanth

and Pope, 2000). The Panel noted that there is less information suggesting that human infants are more sensitive than adults to chlorpyrifos toxicity because of age differences in PON1-mediated detoxication. In humans, blood A-esterase is very low at birth and increases for the first two years of life (Augustinsson and Barr, 1962; Ecobichon and Stephens, 1973; Burlina et al., 1977) or up to 4 years according to more recent data from large cohorts (Holland et al., 2006).

Errors in the metabolism section of the Agency's background materials provided to the FIFRA SAP for this review were pointed out by one Panel member.

> b. There are limited data on the metabolic capacity of pregnant animals and pregnant humans. These limited data on metabolism are supported by some toxicity data in rats. The Agency believes these studies suggest that pregnant animals and humans may be somewhat more sensitive to chlorpyrifos than non-pregnant adults to chlorpyrifos. *Please comment on the Agency's preliminary conclusion and the scientific support for or against this conclusion.*

Panel Response

The Panel agreed with the Agency's preliminary conclusion that "pregnant animals and humans may be somewhat more sensitive to chlorpyrifos than non-pregnant adults" based on metabolic capacity and their effect on levels of key enzymes in modulating chlorpyrifos toxicity. However, panel members noted that only relatively small differences in levels of the key detoxication enzymes involved in chlorpyrifos metabolism have been reported. Some panel members recommended that a direct comparison of the metabolic capacity of pregnant versus non-pregnant females be performed. Other panel members strongly disagreed with this recommendation.

The Panel noted that it is not clear why pregnant females would be more susceptible to the adverse effects of chlorpyrifos than non-pregnant females. The information on toxicity of chlorpyrifos in pregnant animals is limited and the data on biotransformation of chlorpyrifos in pregnant females are inadequate. Reports from the American Association of Poison Control Centers (AAPCC) do not suggest increased susceptibility of pregnant women to chlorpyrifos and other OPs. The physiological response to pregnancy includes an increased vascular volume resulting in relevant hemodilution, along with higher cardiac output and increase in liver weight. These physiological changes may impact liver and serum enzymes involved in chlorpyrifos metabolism that contribute to some extent in the variability of activity observed during pregnancy. Most of the gestational studies published so far report that there is greater concern for the fetus rather than the dam. These studies are inadequate to determine whether the fetus is more sensitive than postnatal pups. The sensitivity to chlorpyrifos clearly decreases when dosing occurs with increasing age in postnatal pups. However, the increased sensitivity does not seem to occur when pups are repeatedly exposed to low doses. In fact, when cholinesterase activity, muscarinic receptor binding, and motor activity changes in response to the antimuscarinic drug scopolamine were compared in juvenile and adult rats treated repeatedly with chlorpyrifos, adults showed more extensive changes (Chakraborti et al., 1993). As noted before, this may be due to more rapid synthesis of acetylcholinesterase molecules in tissues from younger animals

between exposures, thus leading to lesser accumulation of inhibition across exposures in the younger animals.

The Panel discussed the biological significance of the possible changes in metabolic activity during pregnancy relative to expected toxicology outcomes. In humans, the main three CYP450 isozymes that metabolize chlorpyrifos are CYP2B6, CYP2C19, and CYP3A4. The net effect of these isozymes on the balance of desulfuration/dearylation is unknown since CYP2B6, CYP3A4 and CYP1A2 promote the activation of chlorpyrifos to chlorpyrifos-oxon whereas CYP2C19 detoxifies the oxon by means of a dearylation reaction. Whereas, CYP3A4 increases during pregnancy, CYP2C19 decreases (Anderson, 2006) and no data on CYP2B6 in pregnant women are available. Pregnancy-induced decreases in levels of the CYP2B1/2 protein, which is the orthologous form of human CYP2B6, has been reported in rat liver but this is not accompanied by a statistically significant decrease in pentoxyresorufin O-dealkylase (PROD) activity (Czekaj et al., 2000).

Review of the literature indicates that there appears to be a reduction in the activities of several enzymes potentially important in the detoxication of chlorpyrifos associated with pregnancy, including PON1, carboxylesterases and blood cholinesterases. The Panel agreed with the Agency that the "importance of the decreases (in detoxifying enzymes) is unknown at environmental exposures." Although the importance of these decreases under low environmental exposures is not known, decreases during pregnancy may indicate a corresponding reduced capacity to detoxify chlorpyrifos. While the enzymes mentioned above are expected to have lesser (or perhaps no significant) role in modulating toxicity with low level exposures, the limited evidence does suggest a potential for an overall reduced capacity to detoxify chlorpyrifos during pregnancy.

2. Cholinesterase Inhibition (Issue Paper Section 3.2, Appendix B):

The Agency has reviewed numerous studies submitted for pesticide registration and from the literature in animals and human on the AChE-inhibiting effects of chlorpyrifos in blood and in the peripheral and central nervous system.

a. Regarding inhibition of AChE, the Agency has preliminarily concluded that post-natal studies in rat support the conclusion that juveniles are more sensitive than adults. The Agency has further concluded that sensitivity is greatest in younger pups and decreases as pups mature towards adulthood. *Please comment on these Agency's preliminary conclusions and the scientific support for or against these conclusions.*

Panel Response

The Panel agreed with the Agency's conclusion that post-natal rats are more sensitive than adult rats with respect to increased AChE inhibition and lethality following acute exposures. A number of studies have consistently shown that juvenile rats are more sensitive than adults to cholinesterase inhibition and cholinergic signs of toxicity following acute doses of chlorpyrifos exposure. In addition, several studies show a gradient of sensitivity to acute chlorpyrifos in rats during postnatal maturation. This greater sensitivity appears due to a lower detoxication capacity and as these capacities develop, the rats become more aligned with the lower adult sensitivity.

However, this greater sensitivity may be true mainly for high dosages because of the saturation of the detoxication mechanisms. This difference in sensitivity (measured as acetylcholinesterase inhibition) between juveniles and adults is less likely to be apparent at low environmentally relevant dose levels because the detoxication mechanisms will not be saturated. Some on the Panel felt that physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models are likely to be of greatest value in predicting such age-related differences over a span of dose levels.

The Panel, however, did not support the suggestion that post-natal rats are more sensitive than adult rats under repeated exposures. The timing of the measurement of cholinesterase inhibition, as well as, the timing between exposures can be pivotal in the degree of inhibition observed. Results in juveniles can be confounded by their higher capacity to synthesize of new AChE molecules. For example, Chakraborti et al. (1993) reported that juvenile rats are less sensitive than adults under subacute chlorpyrifos dosing (i.e., 40 mg/kg/day, every four days for 4 doses). Four days after the last exposure, adult rats showed 90-92% inhibition of brain cholinesterase while juvenile rats show only 54-59% inhibition. Greater inhibition of brain cholinesterase in adults was correlated with more extensive reductions in muscarinic receptor binding. These receptor changes were correlated with enhanced locomotor responses to the antimuscarinic drug scopolamine in adults only, out to eight weeks after the end of dosing. Thus, under these conditions, adults appear markedly more sensitive to cholinesterase inhibition and cholinergic effects of chlorpyrifos. Liu et al., (1999) report that repeated, daily chlorpyrifos exposures (5 mg/kg/day, for 14 days) were associated with relatively similar degrees of brain AChE inhibition between juveniles and adults 1 day after either the 7th or 14th dose, but more extensive inhibition was noted in adults 8 days after termination of dosing. Relatively similar changes in muscarinic receptor binding were also noted between age groups following daily exposures. Together, these studies illustrate the ability of brain cholinesterase to more effectively recover between repeated doses in pups and the possibility of relatively more extensive cholinergic responses in adults following repeated chlorpyrifos dosing under the same conditions (repeated, "low" dose exposures).

Overall, the Panel decided that a conclusion of greater sensitivity to cholinesterase inhibition in juvenile rats compared to adult rats only holds for acute high exposure situations. When acute exposures occur at environmental concentrations and/or under repeated (low-level) exposures; however, many on the Panel believed that higher sensitivity in younger animals under "environmental concentrations" would be unlikely. Other members stated that this conclusion cannot be made without having a better idea of the meaning of "high exposure level situations" or "environmental concentrations."

b. There are multiple gestational studies available which provide AChE data in dams and/or fetuses. These gestational studies have consistently shown that AChE inhibition observed in the dam is greater than in the fetus. These studies suggest that the dam serves to protect the fetus. However, TK gestational studies have shown that fetal tissues have similar or higher levels of chlorpyrifos and/or its metabolites than the dam. In addition, multiple studies have shown that recovery from AChE inhibition is more rapid in juveniles compared to adults. This rapid recovery combined with production of the AChE enzyme as the rats mature leads to less AChE inhibition observed in the juveniles. The Agency has preliminarily concluded that AChE inhibition data from repeated dosing gestational studies showing less inhibition in fetuses may

not reflect the true potential toxicity to the fetus. *Please comment on the Agency's preliminary conclusions and the scientific support for or against this conclusion.*

Panel Response

The Panel agreed with the Agency's preliminary conclusion regarding observations of lower brain cholinesterase inhibition in pups (rats) compared to dams following gestational OP exposures. However, as noted in the previous question on the findings from the juvenile versus adult studies, the cholinesterase activity measured depends on the time of sampling relative to time of exposure. Whether one considers gestational or postnatal exposures, the longer the time from dosing to measurement of cholinesterase inhibition, the lesser the inhibition expected in younger animals. If the time between dosing and measurement is held constant and the same for dams and pups, one would expect lesser inhibition in pups compared to the dam. This is thought to be due to the more rapid synthesis of new proteins including cholinesterase in tissues of younger animals. Thus, the addition of newly synthesized (uninhibited) cholinesterase molecules in younger individuals contributes to more effective and rapid recovery of activity.

The Panel also agreed with the Agency's preliminary conclusion that "AChE inhibition data from repeated dosing gestational studies showing less inhibition in fetuses may not reflect the true potential toxicity to the fetus." The Panel based its decision after review of those studies indicating the sensitivity to cholinesterase inhibition at dosages of chlorpyrifos sufficient to elicit AChE inhibition. One Panel member cautioned about equating AChE inhibition to toxicity, as is implied in the Agency's question. Inhibition is a sensitive biomarker of exposure and is a precursor to at least some, if not all, of the adverse acute effects of AChE organophosphates. In addition, there can be no cholinergic toxicity without previous AChE inhibition. Lassiter et al. (1998) confirmed less inhibition of brain cholinesterase of pups treated daily with chlorpyrifos (7 mg/kg/day) from GD14-18, when assayed from 2 to 48 hours after the last dose compared to peak inhibition measured at 5 hours after a single dose of chlorpyrifos (10 mg/kg) on GD18. Relatively similar inhibition was noted in both dam and fetus (approximately 40% in the fetus and 50% in the dam) for the acute dose treatment. Hunter et al. (1999) also found that peak AChE inhibition was 5 hours for the dam and fetus and this paralleled the time of peak TCP levels in the brain.

Lassiter et al. (1998) considered several mechanisms for differential ChE inhibition, but concluded that more effective recovery of enzyme activity following each daily exposure in the fetal tissues prevented accumulation of ChE inhibition, while slower recovery of activity in the maternal brain allowed increased ChE inhibition throughout the dosing period. This is essentially the same mechanism described earlier in postnatal pups to explain the lesser cholinesterase inhibition and effects on other cholinergic markers in pups as compared to adults that were treated at subactute doses with chlorpyrifos (Chakraborti et al., 1993). The recovery of inhibited AChE, i.e., the apparently lower level of AChE inhibition in juveniles compared to adults, is very likely due in large measure to greater synthesis of new AChE molecules during growth. In animal studies, it would be unlikely that significant "synthesis of new AChE" would occur in a 5 hour window to a level such that it would alter AChE activity and give the impression of less inhibition. In fact, AChE activity in control animals changed very little between 2, 5, and 10 hours following exposure as reported in Lassiter et al. (1998). Thus, AChE measurements prior

to 5 hours may be an accurate reflection of the amount of AChE inhibition, but AChE measurements beyond that point are subject to question.

The Panel agreed that the timing of the AChE assay following exposure may be less important when evaluating the degree of cumulative inhibition in dams following repeated dosing, but critical when evaluating the more rapid inhibition and recovery in the fetus or pup. A more accurate assessment of AChE inhibition in the growing juvenile could be determined if the timing of the sample collection were closer to the time of peak effect and if more interim sacrifices were used in these experiments. Some on the panel recommended more studies to examine AChE inhibition resulting from lower doses of chlorpyriphos in dams and developing neonates/pups, at more time points, as well as more carefully quantifying AChE protein levels as well as AChE inhibition.

Regardless of whether there was recovery of AChE inhibition or increased AChE synthesis, this event is expected to be of little consequence at levels of exposure that are below those inhibiting the target enzyme. It was pointed out that the human fetus is slower developing than the rat, so rapid synthesis would not be present. Some Panel members felt that in the human fetus there would be no inhibition at all at environmentally relevant levels of exposure. But another member indicated that inhibition might be a concern for exposures experienced by agricultural workers. Many agricultural workers are women and some of those may be pregnant when exposed to higher levels. One Panel member stated that predicting the toxicological effects in humans based on the available data is too risky based solely on animal studies.

3. Laboratory Studies on the Developing Brain (Issue Paper Section 3.3, Appendix C):

The Agency has performed a literature review of *in vivo* and *in vitro* studies on the effects of chlorpyrifos on the developing brain.

a. From a review of laboratory animal studies, the Agency has preliminarily concluded that gestational and early postnatal exposure at sufficiently high exposures to chlorpyrifos can lead to neurochemical and behavioral alterations persisting into adulthood after any initial AChE inhibition has reversed. The Agency has put particular emphasis on the behavioral data because studies are available from multiple laboratories. *Please comment on the Agency's preliminary conclusions and the scientific support for or against this conclusion.*

Panel Response

In general, the Panel was in agreement with the preliminary conclusions made by the Agency. The preliminary scientific findings and conclusions are supported by the review of the *in vivo* neurobehavioral data from multiple laboratories on two mammalian species (i.e., rat and mice) of gestational exposure and/or early postnatal exposure to chlorpyrifos. The Panel concluded that independent of the exact time frame of the gestational or postnatal exposure, only doses of 1mg/kg or greater demonstrate significant effects on behavior. However, only two studies to date (Maurissen et al., 2000; Jett et al., 2001) have examined developmental effects at the lower dose level of 0.3 mg/kg, and neither study is considered sufficient (see below). The Panel noted

that the inclusion of some more recent studies would add to the number of laboratories involved, as well as increase the number of neurobehavioral endpoints examined. One panel member provided a table of studies that supplements the information provided in Table 3 of the Agency's issue paper (see Appendix 1).

Two areas of concern were identified and discussed. The first involved methodological issues in the studies reviewed. The second was conceptual, and involved the use of the phrase "sufficiently high exposures" in the Agency's charge question.

The methodological issues centered on the consistency of behavioral endpoints reported as significant. The Panel noted that although behavioral findings were consistently found, (refer to Table 3, Agency Issue Paper), a somewhat inconsistent set of behavioral changes were reported. In some cases, motor activity is increased and in others, it is decreased. In learning and memory tests, error rate can be increasing, decreasing or not affected under similar treatment conditions. Increased and decreased habituation was reported with different gestational exposure paradigms. Gender differences are also reported in some studies. In studies comparing exposure level, lower dosages sometimes caused greater effects. These inconsistencies likely reflect differences in route of administration, developmental period during exposure to chlorpyrifos, method used to measure changes in the same behavioral domain, and/or choice of dependent variable. For example, motor activity can be measured by distance traveled in an open field and electronically by beam breaks. Moreover, different studies use different types of testing chambers, or number of crossings in an elevated plus maze. The use of several detection methods for motor activity may present difficulty in comparing across studies, especially with respect to younger animals. Concerns were also raised about the high level of variability of measurements and its effect on such endpoints as the mean startle response (see Maurissen et al., 2000).

While the Agency put particular emphasis on the behavioral data, primarily because it is available from multiple laboratories, all related data were considered in identifying long-term changes in the nervous system. With respect to the Maurissen et al. (2000) study, the Panel raised concerns on the use of static morphometrics to evaluate a dynamically changing brain structure. Measurement of the hippocampal dentate granule cell layer at postnatal day 12 would potentially capture a structural assessment of a region with poorly defined landmarks at a time when the neurons are undergoing an active period of migration. In the adult brain, morphometric measurements of the cortical regions displayed about 10% variability, a level expected to be within the normal variability for such crude measurements. Unbiased stereology is recommended by the Panel to address questions of regional volume or cell number.

Several Panel members specifically noted methodological shortcomings in the Jett et al. (2001) study. Although it is only one of two studies to investigate behavioral changes after a dose of 0.3 mg/kg of chlorpyrifos, there were significant problems with respect to the training paradigm i.e., the Morris water maze [MWM]. Animals were not tested to asymptote and only 60% learning was evident in the control group. Neurochemical data indicated that AChE inhibition was not observed at a dose of 7 mg/kg, a finding at odds with other published studies. In addition, behavioral changes were noted for the 0.3 mg/kg/dose only when dosing continued during testing. This represents a confounding factor in determining the acute versus development effects of chlorpyrifos.

The Panel agreed with the Agency's expressed caution about the use of DMSO as a vehicle because of its intrinsic toxicity and influence on absorption. In addition, the "observed signs of discomfort" in pups injected with DMSO reported by Marty et al. (2000), and more recently by Carr and Nail (2008), raised concerns about the use of this vehicle in developmental studies. An additional concern was raised by the Panel concerning the influence of DMSO on the toxicity of the compound under study. Ballough et al (2008) reported that DMSO, administered at levels below those used in many developmental chlorpyrifos studies, enhanced the toxicity of soman by exacerbating the neuropathology caused by soman-induced seizures. The interaction of DMSO and soman, an OP, raised concerns about a possible DMSO-chlorpyrifos (also an organophosphate) interaction in developmental studies. Additional concerns were raised regarding both DMSO and ethanol as vehicles used not only in the *in vivo* studies, but also in the *in vitro* studies.

There was also discussion about the varied routes of administration (ROA) of chlorpyrifos used for developmental exposure. The Panel noted that each ROA used presented drawbacks and most if not all were stressful. It was generally agreed that the subcutaneous ROA was valid for gestational exposure. Moreover, despite a variety of routes used in the literature, there are consistent reports of long-term behavioral changes in offspring after recovery of cholinesterase inhibition.

The Panel noted that many of the papers cited by the Agency do not link exposure periods to chlorpyrifos with structural changes occurring in the developing brain. While the EPA developmental neurotoxicity guideline studies defined a broader period of developmental exposure, more recent studies from the peer-reviewed literature have attempted to demonstrate that there are distinct developmental windows of vulnerability. While effects of chlorpyrifos can be detected in the older animal a direct linkage between the developmental window with regard to the stage of brain development and endpoints examined was ill-defined. It was suggested that a more specific examination of the critical events occurring at the time of exposure and the specific targeted endpoints of interest would be a valuable contribution to the literature. Such data would be particularly important to distinguish cholinergic versus non-cholinergic modes of action for specific adverse effects. Clancy et al. (2007) and the website, http://www.translatingtime.net, were suggested as potential resources for the design of such studies with respect to timing of brain development.

The Panel indicated that the phrase "at sufficiently high exposures" is open to interpretation. Overall, it was interpreted by the Panel in this meeting to refer to studies in which the exposure level was within a dose range expected to inhibit brain AChE. The majority of studies reviewed by the Agency focused on an exposure near 1 mg/kg/day, a level generally sufficient to inhibit brain acetylcholinesterase in most treatment/age groups studied, and which produce behavioral alterations. However, measurements were often obtained at a single time point post-dosing and at a time when the period of peak inhibition may be missed. The availability of dose-response data was limited and in some studies the calculated levels of inhibition were not consistent with the predicted levels of inhibition for high dose exposure. Studies examining critical developmental periods for exposure were primarily focused on dose levels expected to produce AChE inhibition. Given the nature of the gestational exposure studies, the use of fetuses for

neurochemical analysis is limited. Given the critical role of the cholinergic system for brain development, the lack of sufficient data to support the absence or presence of enzyme inhibition is a significant problem in the interpretation of the results from many of these developmental studies. The inherent problems in assessing brain AChE inhibition during development and the type of measurements needed for the assessment were noted in the Agency's document. The Panel noted that it is possible that the morphogenic role of AChE may alter aspects of brain development and neurogenesis, even in the absence of detectable levels of inhibition.

The critical point stressed was that dosages considered "sufficiently high" to elicit neurobehavioral disruption into adulthood would also be anticipated to affect the target enzyme for the common mechanism of toxicity for chlorpyrifos and other organophosphate insecticides. Whether any such changes were initiated by early inhibition of acetylcholinesterase remain unclear.

Errors in the Agency's Issue Paper described below were noted.

1) Section 3.3.1, page 44, paragraph 3. The behavioral effects in the Maurissen et al. (2000) study are only limited to an increased latency to peak response in the auditory startle response that was independent of the dose of chlorpyrifos. The Agency interpreted the data to mean that there was both a decreased amplitude and increased latency at PND 22.
2) Section 4, page 16, paragraph 4.There is no dose-response for effects of ketanserin on reference memory since increased errors were observed in the low and high dosages but not the medium dosage.

b. Consideration of the mode of toxic action is an important component of risk assessment. The International Programme of Chemical Safety (IPCS) and International Life Sciences Institute Risk Science Institute (ILSI RSI) have developed a Mode of Action (MOA)/Human Relevance Framework which provides structure and transparency to MOA analyses (Meek et al., 2003; Seed et al., 2005 and Boobis et al., 2006). IPCS have combined and extended these components to produce a unified Human Cancer Relevance Framework (IPCS HRF). In this approach, involvement of a series of key events in the MOA is established on weight-of-evidence, using criteria based on those described by Bradford Hill, taking account of factors such as dose-response and temporal concordance, biological plausibility, coherence and consistency. Other MOAs that logically present themselves also should be considered. Once an MOA is established, qualitative and quantitative comparison of each key event between the experimental animal and humans enables a conclusion as to likely relevance of the MOA for human risk. In the case of chlorpyrifos, the Agency has considered the available mechanistic data but has not evaluated these data in the context of MOA/human relevance framework. It has been initially determined that there are insufficient data to develop a series of supportable key events (as in a mode of action analysis[1]) for neurodevelopmental toxicities other than AChE inhibition. The Agency notes a particular lack of data on dose response and temporal concordance that are critical in a MOA framework analysis. There may be other mechanisms which lead to effects on the developing brain but a supportable mode of action(s) can not be elucidated at this time. *Please comment on the Agency's preliminary conclusions and whether there is sufficient*

[1] For information on the Mode of Action Framework, see U.S. EPA ,1999, 2005; Sonich-Mullin et al., 2001; Meek et al., 2003 ; Seed et al 2005 and Boobis, et al, 2006

scientific information to merit a full mode of action framework analysis. If a mode of action framework analysis is pursued, what would be the biologically plausible hypotheses to evaluate?

Panel Response

There was a consensus of the Panel that available data were inadequate to support a weight of evidence evaluation for non-cholinergic mode(s) of action for the behavioral alterations following gestational and early postnatal exposure to chlorpyrifos that persisted into adulthood. The Panel agreed that the available information does not allow for behavioral endpoints to be considered as a point of departure and recommended, based upon currently available data, that cholinesterase inhibition be used as the PoD.

Pointed out at the beginning of the discussion of this charge question was the fact that a mode of action/human framework analysis requires consideration of testable hypotheses with identified key events in a causal pathway. It then requires comparison of systematic analyses of traditional criteria for weight of evidence for each of these hypotheses including consistency, dose-response and temporality among early key and end events, biological plausibility and coherence; all of which requires expert multidisciplinary input. The framework, therefore, focuses attention early on key events that can be compared between species and how they are quantified, ultimately critical points in driving the dose-response relationship. While the structured analysis contributes to increased transparency in weight of evidence in risk assessment, it is also helpful in framing research relevant to risk assessment and permits iterative dialogue between the research and risk assessment communities, as a basis to generate more appropriate data.

The data presented in Appendix C of the Agency's issue paper summarizes the studies on persistent behavioral changes in adults following gestational and early postnatal exposure as well as selected *in vivo* and *in vitro* studies on the interaction of chlorpyrifos with several neurochemical parameters. The Agency noted in its issue paper that during development of the brain, acetylcholine plays a role in morphogenesis, and AChE may alter aspects of neurogenesis in the absence of a detectable level of inhibition using current methods. The Panel indicated that there is a large amount of variance presented in many of the studies, a lack of dose response for many of the proposed targets, and a lack of reports of altered targets from more than one lab. The Panel concluded that these studies were not inclusive, coordinated, nor conducted in the context of hypothesis testing for mode of action. Rather they were conducted principally for generating testable hypotheses. Without clearly defined and testable hypotheses we are left with uncertainty about whether the long term behavioral changes occur downstream from AChE inhibition or independently of it.

Specific points were raised regarding the question of mode of action. In Figure 5 of the issue paper, the Agency cites Slotkin et al. (2006) to suggest possible mechanisms for how chlorpyrifos may elicit neurodevelopmental effects. The Panel did not find enough information from the Slotkin et al. (2006) paper to determine if chlorpyrifos directly interacts with the molecular components of the proposed pathways that could then lead, in a causal manner, to subsequent neurobehavioral changes. Panel members determined that it was generally unclear in what way the animal studies exhibit "qualitative similarities" to findings reported in children. The animal studies do report neurobehavioral changes in rats and mice following prenatal or

postnatal chlorpyrifos exposures at levels that would be expected to inhibit cholinesterase. These "sufficiently high" exposures may lead to persistent neurobehavioral effects under some conditions, but "qualitative" similarities to neurodevelopmental findings reported in children are difficult to equate.

There are several facets of data evaluation that may need to be examined with respect to effects on serotonin, G-proteins, macromolecules, neurotrophic factors etc. by developmental exposure to chlorpyrifos. For the most part, the effects seen on these systems following chlorpyrifos exposure are modest, and many represent less than a 25% change from control values/levels. These changes were often sex specific and dependent upon the time of exposure and measurement. Although there is always the possibility that such modest changes in neurochemistry and biochemistry will have a significant impact on behavior, replication and validation across laboratories are required. Some Panel members agreed with the Agency's conclusion that, given the effects on learning and memory, characterization of glutamate N-methyl-D-aspartic acid (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors would be important endpoints in assessing the developmental neurotoxicity of chlorpyrifos.

The use of morphometric measurements of brain regions was questioned with regard to age, i.e., regional measurements were conducted during an active period of brain development resulting in the lack of discrete structures due to neuronal migration. The diffuse structural features at early postnatal ages, e.g., PND12, would confound the ability to obtain an accurate measurement. In addition, in the mature animal a change within the range of 10% for such a crude measurement would be expected to be within the normal variability of control tissue. One panel member indicated a preference for the use of unbiased stereology in obtaining structural measurements of discrete brain regions. However, this approach would also be hindered during early postnatal development. Unbiased stereology is a well established method for determining cell number and tissue volume and has been used in developmental, aging, and pharmacological studies. An additional concern was raised in that changes often occur in the network organization of the brain rather than gross cell death. A number of different neural and vascular cell types can be influenced by acetylcholine during development and such effects may not involve cell death but rather a structural or functional alteration. While standard histological assessement of hemotoxylin and eosin stained sections accurately detect cell death and general organizational features of the brain, neither it nor morphometric measurements would detect less than gross cell loss occurring prior to such assessment. Nor would such a standard approach detect changes in the non-neuronal cells within the brain, such as the various glial populations. Additionally, developmental neurotoxicity can be associated with changes in the synaptic organization of the various brain regions and the normal temporal pattern of apoptosis and synaptic pruning, differentiation of radial glia, neuronal migration, synapse stripping, maturation of the resident brain monocytes, the microglia, and the brain vascular system. Thus, to determine low-dose effects for any chlorpyriphos will require more sophisticated methods of analysis.

One Panel member took issue with the material in Section 9: *Problems with studies from the literature*, and in particular with Paragraph 5, Page 28 (see Agency's issue paper) which states "An aspect of some of the *in vivo* studies discussed above is that no AChE inhibition was detected during the windows of exposure at the dose level used. This is especially true for

gestational exposures. This suggests that some other target was affected by chlorpyrifos or its oxon." As stated repeatedly in Appendix B, AChE activity increases rapidly in gestational and neonatal animals and, in many studies, especially the gestational studies, the time of assay does not coincide with the time of peak inhibition. Thus, unless a time course of AChE inhibition is performed and clearly demonstrates that there is no AChE inhibition, the claim of no inhibition or low levels of inhibition may not be accurate.

Some panel members suggested that the recent review of chlorpyriphos toxicity by Eaton et al. (2008) provided a basis for considering possible non-cholinergic modes of action. The serine hydrolase enzyme KIAA1363 is important in an ether lipid signaling network involving platelet activating factor, and is highly expressed in cancer cells. *In vitro* studies demonstrated that this enzyme was inhibited by chlorpyrifos-oxon ($IC50^2$ = 8 nM) within the range of AChE inhibition. i*n vivo*, a 98% inhibition of AChE and lethality due to chlorpyrifos-oxon administration resulted in a 39% inhibition of brain KIAA1363 (Nomura et al., 2006). The relative changes have not been reported for lower dose levels thus, a direct causative role in the toxicity of chlorpyrifos has not been identified. So while KIAA1363 may serve as an important detoxication mechanism, as is supported by the increased toxicity in knockout mice (Nomura et al., 2006), it does not necessarily indicate that this enzyme is a target for "mode of action."

At dose levels significantly lower than any previously reported effects, chlorpyrifos induced the phosphorylation of cAMP response element binding (CREB). This transcription factor plays a role in synaptic plasticity and in cell survival and differentiation. In primary cortical or hippocampal neurons, pCREB was elevated following dosing with chlorpyrifos, 0.06 nM and 1-10nM, respectively (Schuh et al., 2002). No effect was observed in primary cortical astrocytes. Schuh et al. (2002) speculated that, rather than indicative of neurotoxicity, the elevation in pCREB represents a neuroprotective response to metabolic stress in neurons. Some Panel members felt that the studies cited in Eaton et al. (2008) (specifically Table 14 and related text) could be useful to the Agency in evaluating potential alternative modes of action.

Collaboration between EPA and developmental neurotoxicologists may help define research needs for identifying alternative neurotoxic processes involved in the developmental neurotoxicity of chlorpyrifos. At a minimum, such studies would require testing multiple doses of chlorpyrifos between 0.05 and 1.0 mg/kg to set the high dose for expected AChE inhibition. Such data can then be used to determine level of cholinesterase inhibition and related changes in brain development. This may require additional methods development for detection of targeted brain regions or cellular localization. The data set can then be used to identify cholinesterase inhibition or an alternative mode of action for developmental neurotoxicity of chlorpyrifos. Low dose level effects reported for *in vivo* developmental effects require replication as well as the extremely low-dose *in vitro* activation of CREB. Inclusion of data on multiple carboxylesterase activities and in localized brain regions are major requirements for future studies.

[2] IC50 (Isolated Cortical) refers to the concentration of chlorpyrifos in cell culture that produced the observed effect (in this case an increase in CREB) in 50% of the neurons.

4. Epidemiology Studies in Children and Mothers (Issue Paper Section 3.4, Appendix D):

The Agency has evaluated epidemiology studies from three major cohorts: the study sites are: (1) Columbia University, NYC, (2) Mt Sinai, School of Medicine, NYC, both with multi-ethnic urban low income women and infants, and (3) University of California at Berkeley (Center for Health Assessment of Mothers and Children of Salinas, CHAMACOS) with women and their children from farm worker populations.

a. The Agency believes that all three studies provide valuable information on the effects in children of high exposures to pesticides, particularly OPs. For purposes of evaluating human health effects of chlorpyrifos, the Columbia University studies provide more robust information for evaluating the human health effects of chlorpyrifos because it measured chlorpyrifos rather than a metabolite in both environmental (air) and biologic media (maternal and cord blood) and showed that chlorpyrifos was significantly associated with birth outcomes (low birth weight and length) and neurodevelopmental outcomes that were no longer present when the residential uses were cancelled (i.e., conducted a pre- and post-residential cancellation analysis). Although the results reported by the Mount Sinai group are informative with regard to evaluating the relevance of PON1 status in health outcomes, this study is limited because the neurodevelopmental outcomes were linked to non-specific OP maternal urinary metabolites (DAP, DEP and DMP), rather than the chlorpyrifos-specific metabolite TCP. The exposure of the CHAMACOS to many OPs reduces its usefulness in the chlorpyrifos risk assessment because the outcomes can not be specifically linked to chlorpyrifos exposure. *Please comment on the Agency's preliminary conclusions on each of the three cohorts regarding the degree to which the data informs the chlorpyrifos human health risk assessment. Please also comment on the scientific support for or against these preliminary conclusions.*

Panel Response

Overall, the Panel agreed with the Agency's conclusion that although each of the three studies (Columbia, Mt. Sinai, and Berkeley) provide valuable information, the Columbia study is the most robust and appropriate for informing risk assessment with respect to the exposure (chlorpyrifos), outcomes (birth and neurodevelopmental), and population (neonates and children) being addressed by the Agency's evaluation. To be precise, the Columbia study was not necessarily a more robust epidemiological study, but it directly measured chlorpyrifos exposure as opposed to the Mt. Sinai and Berkeley studies where metabolites not specific to chlorpyrifos exposure were measured and reported, including those of other OPs and carbamates. The use of the term "high exposure" in the phrasing of the question was questioned because although the exposures in these studies may be high for residential settings, they are not high when compared to agricultural worker and pesticide handler populations.

The Panel noted that the conclusions of the Agency are supported by the following information:

- All three studies are prospective cohorts with relatively low loss to follow-up and are conducted by respected researchers using widely accepted protocols with results published in peer-reviewed journals.

- The Columbia study has the strength of having used blood measures (both maternal and cord) of chlorpyrifos specifically as opposed to the non-specific urinary metabolites (e.g., diethylphosphate (DEP) and dimethylphosphate (DMP)) utilized in the other two cohorts. There is also high correlation ($r=0.76$; $p<0.0001$) between maternal and cord blood chlorpyrifos levels noted in the Whyatt et al. (2003) study. In addition, the Columbia study has exposure measurements from air monitoring which correlate with both maternal and cord blood (Whyatt et al., 2003).

- A particularly convincing strength of the Columbia cohort is that it spans the residential cancellation date allowing the researchers to evaluate changes in birth and neurodevelopmental outcomes pre and post chlorpyrifos residential cancellation. It was noted that although the pre and post analysis validates exposure measurements, the overall results are more interesting epidemiologically because it looks at the entire spectrum of exposure.

- The initial reference group for the birth outcomes (those whose chlorpyrifos levels in cord blood were below the level of detection (LOD) with similar demographic and socioeconomic characteristics) in the Columbia study was also judged to be appropriate.

- The methodology for exposure measurement was considered good by the Panel and the quality assurance and quality control procedures used by the Centers for Disease Control (CDC) are extensive. The Columbia study included both environmental and biological measurements of chlorpyrifos and biological measurements were made in both maternal and cord blood. It was noted that there are concerns about values near or below the detection limits; however, the precision of the data are acceptable while accuracy is reduced. In other words, the Panel felt confident that the chemical was present when detected in a sample although the reported value may be a poor measure of the actual concentration. The difference between the true and measured amount could be appreciable.

- Rauh and Whyatt presentations to the Agency in 2007 and 2008 (presentations found in the docket, EPA-HQ-OPP-2008-0274) showed intelligence quotient (IQ) results at five years of age and indicated intent to extend the follow-up for this cohort until age 7. This work provides the longest follow-up to date for a population of children who were exposed to chlorpyrifos *in utero*.

The Panel concluded that the Columbia study is epidemiologically sound and that there is minimal selection and information bias. Additionally, any information bias would likely be non-differential since any misclassification of outcome is unlikely to depend on exposure status and misclassification of exposure is unlikely to depend on outcome status. However, even the best-designed epidemiology studies are susceptible to weaknesses inherent to observational studies, and a few of these possible weaknesses are addressed below:

- The possibility of residual confounding in the analysis of the Columbia study data on birth outcomes (Whyatt et al., 2004) was discussed. In particular, variables such as

presence of prenatal care, sexually transmitted diseases, history of substance abuse and of cigarette smoking, low pre-pregnancy body mass index (BMI) and inadequate weight gain are potential confounders. Review of the model covariates found that a complete list of standard variables which are commonly available were controlled for in the analysis including passive smoking, ethnicity, parity, maternal pre-pregnancy weight, weight gain, newborn gender, and season of delivery. In addition, the Columbia cohort was restricted to exclude women who smoked during pregnancy, used illicit drugs, had human immunodeficiency virus (HIV), or had their first prenatal visit after the 20th week of pregnancy (Whyatt et al., 2004). The Panel agreed that these factors limit the possibility of residual confounding. In addition, the authors of the Columbia study further addressed the potential for residual confounding by including a variety of other variables in the analysis. The results as presented in the Rauh et al. 2008 paper are stable and not sensitive enough to eliminate the various confounders. Although there may be other variables not included in the analyses that are associated with either exposure or outcome, in order for a variable to be a confounder it must be associated with both exposure and outcome.

- The Panel also noted that the infants in the Whyatt study were of normal weight. The Panel questioned the significance of lower birth weights and lengths in infants more highly exposed to chlorpyrifos. Low birth weight is defined as a weight of 2500 grams (5 pounds 8 ounces) or less. Low birth weight infants are known to be at increased risk for serious health problems as newborns, lasting disabilities and death (March of Dimes; http://www.marchofdimes.com/professionals/14332_1153.asp. Accessed September 21, 2008). Although infants most affected by chlorpyrifos exposure in the Whyatt study fell within normal parameters for birth weight, there could still be cause for concern. Infants who are born smaller than expected by their genetic potential would have increased risk of perinatal mortality, neurological morbidity and morbidity in general as compared to infants born at their predicted birth weight (Figueras et al., 2007). Reductions in birth weight are also associated with marked increase in chronic diseases in adulthood including diabetes, cardiovascular disease, stroke, and hypertension (Barker, 2004). One issue of concern is whether chlorpyrifos' effect on reducing birth weight would result in more infants falling into the category of low birth weight.

- Concerns raised by public commentators before the Panel about neurobehavioral testing, including the specific tests used, the method of administration, and the training of the individuals administering the test were discussed. The Panel concluded that the Columbia study authors used neurobehavioral tests that are widely accepted in the scientific community and used experienced and well-trained examiners to administer the tests. The Panel also concluded that the results obtained were probably as good as could be obtained given all of the limitations associated with neurobehavioral testing.

- Concerns with the three year period between the sampling of cord blood and the neurobehavioral findings in Rauh et al. (2006) were discussed. The exposure classification is based on a one-time measurement that provides a snapshot of exposure and should not be taken as an absolute representation of total chlorpyrifos exposure throughout pregnancy. Many researchers have argued that chlorpyrifos in blood and

adipose tissue are in steady state (see Appendix I, p. 25, Eaton et al., 2008) so that cord blood provides a reasonable dosimeter for the amount transferred to the fetus. This assertion does not take into account the changes produced in lipid metabolism during pregnancy. In spite of maternal fat stores accumulated in early and mid pregnancy, in late pregnancy human chorionic somatomammotropin (HCS) promotes lipolysis and fat mobilization (Butte, 2000) to meet the increased fetal demand at that time; therefore, fat reservoirs are gradually emptying of the accumulated chemicals. Accordingly, the single measurement of cord blood chlorpyrifos may not be representative of the total exposure during pregnancy, but only reflects exposure happened in the few days before delivery. The Panel noted that exposure events, whether chemical or environmental, that could have occurred in these households over the three year time period are not known. These unmeasured exposure events could influence the neurobehavioral test results, especially given the fact that neurodevelopmental deficits may be multifactorial in origin. It would be important to ascertain whether there are statistically significant differences in neurodevelopmental outcomes at 3 years of age between children with chlorpyrifos levels in cord blood below the LOD and those with a chlorpyrifos level of 6.17 pg/g. Rauh et al. (2006) stated that the Bayley Scales of Infant Development scores (Mental Development Index (MDI) and Psychomoter Development Index (PDI)) were similar in both groups of children. If so, chlorpyrifos levels in cord blood at the time of delivery would not be expected to be associated with neurodevelopmental outcomes.

- It is difficult to compare exposure levels in the Columbia study with the US population (as measured in the National Health and Nutrition Examination Survey (NHANES) or with other biomonitoring studies since urinary metabolite levels were not measured.

- Although the data on post-ban declines in exposure are compelling, limitations must be kept in mind when using these results in the weight of evidence. The study was not designed to assess the effect of the ban, so data are essentially cross-sectional (i.e. exposures among the same women were not measured over time). Additionally, the data presented for exposure levels by year have been crude in nature. It is not likely to make a large difference, but an analysis should be conducted looking at exposure level as the dependent variable and year (an ordinal variable) as the primary independent variable, while also adjusting for any factors that may be associated with both exposure levels and year of study.

- There was discussion about the cut-off values and exposure groupings used in the Columbia studies (Rauh et al., 2006; Whyatt et al., 2004). There was also discussion about the numbers in the various exposure groups in 2004 compared to 2006. Concern was expressed that loss to follow-up may have occurred in more severely affected children or less severely affected children as opposed to a non-differential loss. Some felt that more attention should be given to the sensitivity of the results to the selected "highly exposed" group. For example, the authors said these groups were first based on tertiles, but according to Rauh et al. (2006) the groups were unequal in size. The authors did clarify via personal communication on September 18, 2008 (found in the docket, EPA-HQ-OPP-2008-0274) that these groups were unequal in the 2006 study because only children who reached three years of age were analyzed, suggesting that the original exposure groupings were not balanced. Some panel members noted that the Agency

should explore this further as the loss of children in the high exposure group could not be due to length of follow up as all the children in the high exposure group (bar one) had to have been born prior to 2001 (and hence should have been at least three years old) as after the ban there was only one child in the high exposure group. The Panel felt that it would be useful if the authors could provide additional details on the loss to follow-up in each of the four exposure groups and provide additional details on how the exposure groups were combined. However, it was also noted that the inclusion of both the children below LOD and with low exposures values into the reference group would be expected to attenuate any association, if indeed there is an effect at such low exposures. However, in Rauh et al. (2006), there is no suggestion of such an effect as the most highly exposed group and the group with values below LOD had lower mean MDI and PDI scores than did the two middle levels. There was disagreement on the Panel about the mode of action; therefore, there is no compelling reason to believe that the epidemiologic data should reflect either a linear association or a threshold effect.

- The post-ban declines in effect are based on only one subject being classified as highly exposed in post-ban years. This analysis lacks statistical power and may be sensitive to the cutoff value chosen to classify subjects as highly exposed. It would be interesting to see what the relationships looked like among more equally sized exposure groups that separate between the lower exposure levels.

- The Panel felt that it might be helpful to have a longitudinal component to the Columbia study to determine if the adverse effects persisted in those children who exhibited poor birth outcomes or neurobehavioral deficits.

- It would be useful to examine the results of a statistical analysis that includes all three AChE-inhibiting insecticides in the analysis model as dichotomous variables (above or below LOD) in combination with continuous measurements for these variables. This type of analysis would likely not change the results, but it could be helpful in illustrating threshold or dose response effects.

Despite the questions and discussion noted above, it was affirmed that the Columbia study was indeed quite strong and provided extremely valuable information. Most of the points addressed above are points of clarification or points that if addressed could strengthen the analysis and provide the Agency with better data to use in the risk assessment process.

Given the paucity of human epidemiological studies of organophosphates in children, the other two cohorts (Mt. Sinai and Berkeley) should not be completely discounted. Both studies provide information on abnormal reflexes in neonates, and this data is not available in the Columbia cohort. Additionally, the Mt. Sinai cohort considers PON1 as a factor in the analyses, something that may have some relevance to risk assessment (Engel et al., 2007). More information may be gleaned from the Berkeley cohort (Eskenazi et al., 2004). A close comparison of OP metabolite concentrations in this study with those reported in NHANES may be useful. It is interesting to note that median 3,5,6-trichloro-2-pyridinol (TCP, a specific metabolite of chlorpyrifos) levels in this cohort were similar to those reported in NHANES; whereas, DEP (diethylphosphate, one of the non-specific urinary metabolites) levels were much higher. This suggests chlorpyrifos

exposures in the study are similar to "background level" and that exposures to other OPs (besides chlorpyrifos) that are metabolized to DEP are proportionally much higher in this cohort (relative to chlorpyrifos) than the general population. Other OPs may very well be driving the associations between DEP/DMP (non-specific organophosphate urinary metabolites) levels and adverse outcomes. However, because chlorpyrifos is likely to contribute very little to the overall DAP levels in the Berkeley cohort TCP and DAP levels may be poorly correlated. Thus, in the TCP analysis, it may (with some caution exercised) be possible to additionally adjust for DAPs in an attempt to assess more chlorpyrifos (and/or chlorpyrifos-methyl) specific associations. The presence of these other OP pesticides may overshadow any TCP associations, but may still be worthwhile to investigate.

The Panel acknowledged that there are potential confounders and issues that reduce the utility of both the Mt. Sinai and Berkeley cohorts for risk assessment. For example, both studies measure organophosphate metabolites in urine but chlorpyrifos is not specifically measured. The Berkeley study, in particular, has the least relevance to chlorpyrifos risk assessment because only a small percentage (10%) of the pesticides applied in the Salinas Valley are chlorpyrifos; therefore, the Panel assumed that chlorpyrifos would make only a small contribution to the non-specific metabolites measured in the study, and as a result might be expected to have small impact on the study outcomes, although this assumption has not been verified. As such, it is difficult to ascribe the effects seen to chlorpyrifos, in particular, rather than OPs in general.

A recently published study by Samarawickrema et al. (2008) entitled *Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka* was brought to the attention of the Panel and suggested for use in clarifying the issue of whether maternal and cord blood are differentially distributed to the fetus. It was suggested that the results from this study could be compared to the results observed in Rauh et al. (2004) and Mattsson et al. (2000). Not having seen the Samarawickrema et al. study, the Panel could only recommend that the Agency consider this study when this issue is revisited. The Panel also encouraged the Agency to identify and review any other published studies on this topic, even if of lesser size and scope, since they may also contribute to the total weight of evidence analysis of the potential neurobehavioral effects of chlorpyrifos.

b. Data from Whyatt et al. (2003) show that 100% of air samples detected three AChE inhibiting pesticides (chlorpyrifos, diazinon and propoxur). Similarly, all three pesticides were found in 48-49% of umbilical cord samples at lower levels than chlorpyrifos. The investigators reported that chlorpyrifos was significantly associated with decreased birth weight and length, even after statistically controlling for these two OPs; a similar analysis has not been conducted for the neurodevelopmental outcomes. The Agency can not rule out that exposures to all three AChE-inhibiting pesticides in combination resulted in the neurodevelopmental health outcomes reported in the studies. However, this possibility does not rule out the potential role of chlorpyrifos in contributing to the reported health outcomes, particularly given the reported findings pre- and post-voluntary cancellation. In balance, given, that 1) measured levels of chlorpyrifos have been statistically associated with multiple birth and neurodevelopmental outcomes; 2) these associations are correlated in time prior to the cancellation of indoor uses of chlorpyrifos when exposures were much greater (and thereby show some degree of dose-response); and 3) there are animal data which support neurobehavioral effects resulting from

gestational exposure, the Agency has preliminarily concluded that chlorpyrifos likely played a role in these outcomes. *Please comment on the Agency's preliminary conclusion and the scientific support for or against this conclusion.*

Panel Response

Overall, the Panel agreed with the Agency's conclusion that chlorpyrifos likely played a role in the birth and neurodevelopmental outcomes noted in the three cohort studies. The Panel agreed with the Agency on the following points: a) exposures to all three AChE-inhibiting pesticides in combination cannot be ruled out as contributing to the birth and neurodevelopmental outcomes, b) the potential of combination and/or additive effects of these three compounds does not rule out the role of chlorpyrifos in contributing to the outcomes, and c) it cannot be stated that chlorpyrifos is the sole contributor to the observed outcomes. These conclusions are supported by the effects observed at various age groups across the three cohorts as summarized in Table 1.

1) **Strength and significance of association** as reflected by the following results from the Columbia cohort:

a. Extremely large odds ratios (OR) for attention disorders (OR=11.26; 95% CI: 1.79-70.99), attention deficit hyperactivity disorder (ADHD OR=6.50; 95% CI: 1.09-38.69), and pervasive developmental disorder (PDD OR=5.39; 95% CI: 1.21-24.11) were seen when comparing high to low chlorpyrifos exposure groups (Rauh et al., 2006). Although limited sample sizes resulted in fairly large confidence intervals, the magnitude of these results is so large that they are unlikely to be affected by residual confounding.

b. There were statistically significant deficits in birth weight of 150 grams when comparing high exposure to exposure that was below the level of detection (LOD); and, decreases of 43 grams in birth weight per log unit increase in chlorpyrifos in cord blood. Statistically significant deficits in birth length were also noted (Whyatt et al., 2004).

c. There were statistically significant deficits of 6.5 points on PDI at 3 years of age when comparing high to low exposure groups (Rauh et al., 2006).

d. There were 2.4 times increased odds (95% CI: 1.1-5.2) of mental delay and 4.5 times increased odds (95% CI: 1.6-12.7) of psychomotor delay when comparing high to low exposure groups at age 3 (Rauh et al, 2006).

e. There were deficits of 5.6 points on verbal IQ and 5.1 points on full IQ at age 5 (Whyatt, EPA presentation, 2007).

2) **Consistency of association** between the three cohorts is that they all found some developmental effect in a population exposed to elevated levels of OPs. The Berkeley and Mt. Sinai cohort studies measured non-specific urinary DAP metabolites (e.g., diethylphosphates (DEPs), dimethylphosphates (DMPs) produced by many OP pesticides including chlorpyrifos. However, urinary levels of 3,5,6-trichloro-2-pyridinol (TCP) were not measured in these two studies, but only in the Columbia cohort study. Therefore, the conclusions below are based on the non-specific DAP metabolites rather

than the more specific indicator, TCP. The Panel noted, however, that urinary TCP does not absolutely correlate with exposure to chlorpyrifos, but could be an indicator of exposure to other pesticides including methyl chlorpyrifos and the herbicide trichlopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid, Remedy®) or even to TCP itself (as an environmental degradate of chlorpyrifos, methyl chlorpyrifos or trichlorpyr). However, developmental effects were consistently observed in children whose mothers exhibited biomarkers of exposure to OPs in each of these studies.

a. There were increased abnormal reflexes in neonates associated with maternal urinary DAP measurements in both the Berkeley and Mt. Sinai cohorts (Young et al., 2005; Engel et al., 2007).

b. There were increases in MDI associated with increases in DEP metabolites at 1 year (Eskenazi et al. 2007). Although Eskenazi and colleagues found decreases in MDI associated with prenatal DEP metabolites at 2 years, they were not statistically significant (see Table 4, Eskenazi et al., 2007).

c. Results from cohort studies conducted by Eskenazi. et al. (2007) indicated total urinary prenatal DAP metabolite measurements were associated with increased odds ratios of pervasive developmental disorder (PDD) at 2 and 3.5 years (Eskenazi et al., 2007) and child urinary DAP metabolites were associated with increased odd ratios of PDD at 2 and 3.5 years (Eskenazi et al., 2007; Eskenazi et al., in preparation as cited in the Whyatt presentation made to EPA in 2007).

d. Results indicated prenatal urinary DAP measurements were associated with decreased verbal IQ and full-scale IQ at 3.5 years and decreased verbal IQ at 5 years (Eskenazi et al., in preparation, Whyatt presentation to EPA in 2007). However, since DMP is definitely not a metabolite of chlorpyrifos, the associations stated here and in all of the previous points must be viewed with caution so as to not overinterpret the significance of the finding

3) A **crude dose-response relationship** as evidenced by the following results from the pre and post cancellation analyses in the Columbia cohort:

a. There were statistically significant deficits in birth weight (211 grams when comparing high to LOD groups) in children born before the residential cancellation, but there were no significant deficits in these outcomes in children born after the residential cancellation (Whyatt, EPA presentation, 2008).

b. Statistically significant decreases in birth weight and birth length per log unit increase of cord blood chlorpyrifos were evident in children born before residential cancellation but the association was not significant for children born post-cancellation (Whyatt et al., 2004).

c. There were statistically significant reductions in maternal personal air samples and cord blood chlorpyrifos levels pre- and post-cancellation and a statistically significant

reduction in the numbers of newborns who were categorized in the highly exposed group (Whyatt et al., 2004).

d. There were statistically significant increases in maternal personal air samples and cord blood chlorpyrifos levels in 3 year MDI and PDI scores when comparing pre-cancellation to post-cancellation periods (Rauh et al., 2006).

4) **Persistence of strong statistically significant associations** after controlling for the effects of other compounds as evidenced by the following:

a. Additional analyses on birth weight and neurodevelopmental outcomes (MDI and PDI) controlling for diazinon did not reduce the effect of chlorpyrifos (Whyatt, August 12, 2008, personal communication).

b. Association between birth weight and length remained statistically significant after controlling for diazinon and isopropoxyphenol (Whyatt et al., 2004). The authors noted that blood concentrations for chlorpyrifos were higher than those for diazinon and propoxur. The Panel had some questions about these calculations.

Table 1. Neurodevelopmental Effects with Chlorpyrifos and Its Metabolites and the Metabolites of OP Insecticides in Three Epidemiological Human Cohort Studies
Source: Appendix D, USEPA; Eaton et al., (2008), referenced studies

Age	CHAMACOS (Non-specific OP metabolites + TCP measured)	Mt. Sinai (Non-specific OP metabolites measured)	Columbia (chlorpyrifos, TCP, propoxur, diazinon)
Neonate	Abnormal reflexes (maternal urinary DAPs): During pregnancy, total DAPs (summed DEP metabolites and DMP metabolites) with increased number of abnormal reflexes and presence of 3 or more abnormal reflexes. (BNBAS: Brazelton Neonatal Behavioral Assessment Scales) (Young et al., 2005)	Abnormal reflexes (maternal urinary DAP metabolites): Increased total DAPs, (summed DEP and DMP metabolites) with increased proportion and number of abnormal reflexes (BNBAS) (Engel et al., 2007)	
6 months	No association with prenatal TCP and MDI (Motor Development Index)/PDI (Psychomoter Development Index) (BSID-II: Bayley Scale of Infant Development – II) (Eskenazi et al., 2007)		
1 year	• Child DEPs associated with increases in MDI (BSID-II) (Eskenazi et al., 2007) • No association with prenatal TCP and MDI/PDI (BSID-II) (Eskenazi et al., 2007)		
2 years	• Total urinary prentatal DAP metabolites associated with increases in PDD (Psychomoter Development Delay) (CBCL: Child Behavior Checklist)) (Eskenazi et al., 2007) • Child DAPs (measured DMPs, and DEPs)associated with increase in PDD (CBCL) (Eskenazi et al., 2007) • Prenatal DAPs (measured DMP, one DAP) associated with decrease in MDI (BSID-II) (Eskenazi et al., 2007) • Child DAPs (measured DMPs) associated with increases in MDI (BSID-II) (Eskenazi et al., 2007) • No significant association of TCP with MDI or PDI. (BSID-II) (Table 6, Eskenazi et al., 2007)	Prenatal DAPs associated with decrease in MDI (BSID-II) (Engel et al., in prep)	
3 years	IQ deficits associated with prenatal DAPs at 3.5 years (Ezkenazi et al., in prep)		Increased chlorpyrifos with decreased mean PDI, increased percent with cognitive (MDI) and motor problems (PDI), increased risk of mental and motor delay, increased problems with attention and ADHD (Attention Deficit Hyperactivity Disorder) and PDD. Decreased levels of chlorpyrifos after January 2001 associated with improved scores on MDI and PDI. (BSID-II and CBCL) (Rauh et al., 2006)
5,7 years	IQ deficits associated with prenatal DAPs (Ezkenazi et al., in prep)		IQ deficits associated with chlorpyrifos (Rauh 2008, presentation at SAP meeting, see Docket: EPA-HQ-OPP-2008-0274)

Some caveats and areas for further analysis and clarification were noted by various panel members:

1) In the Berkeley cohort, there was a finding of increased head circumference with DEP levels (3 DEPs were measured) in maternal urine and increased length and head circumference with DMP levels (3 DMPs were measured) (Ezkenazi et al., 2004). This could be a chance finding or it may be due to the exposure profile in this cohort. However, the Mt Sinai study found an association between decreased head circumference and maternal urinary TCP levels above the limit of detection (Engel et al., 2007). Due to the nature of the exposure in the Berkeley cohort, it was noted that perhaps these urinary levels do not reflect significant exposures to chlorpyrifos given that DAP metabolites have two potential origins: breakdown of products arising from OPs in general and direct absorption from environmental sources and diet. As measurements of DAP metabolites are non-specific in nature, the particular chemical involved may be one or more of diethyl phosphate insecticides in use. With regard to the Mt. Sinai study, the results indicated that maternal urinary TCP levels reflected not only direct chlorpyrifos exposure, but also exposure to TCP residues directly from the environment or diet. In addition, the Panel noted that PON1 polymorphism may potentially affect those urinary metabolite levels because women with the higher activity phenotype will eliminate higher levels of TCP in urine as compared to those with the lower activity phenotype. However, this statement is not true if exposure levels are low and below enzyme saturation in the case of carriers of a low activity phenotype.

2) In interpreting the Columbia studies, the Agency should bear in mind that diazinon and propoxur as well as other unmeasured potential developmental neurotoxicants were present along with chlorpyrifos, and the exact roles of all of these components in the mixture cannot be determined. In Table 3 of Whyatt et al. (2004) it is seen that the beta coefficients for the association with birth weight are -42.6 for chlorpyrifos and -44.2 for diazinon. The beta coefficient for chlorpyrifos is statistically significant while the coefficient for diazinon shows large variability and is not statistically significant. One panel member questioned whether selecting chlorpyrifos over diazinon is biologically justified although it may be statistically justified. When the effects of chlorpyrifos and diazinon are summed using U.S. EPA methodology (U.S. EPA, 2001), there is a slightly greater reduction in birth weight (b= -49.1). This may indicate that the effect of the combined chemicals is slightly greater than the individual chemicals alone and that there could be potential interaction between the two chemicals with respect to the association. Given these results, some on the Panel questioned whether one should single out just one of the two chemicals when both are present and there is a potential interaction. Rauh et al. (2006) did not consider diazinon or mixtures in the neurobehavioral study but justified the selection of chlorpyrifos based on the birth weight data, ignoring the similarities of diazinon with chlorpyrifos and the significance of the mixtures. There was disagreement among the Panel with this interpretation. Confidence limits as well as the beta coefficients must be considered when interpreting statistical analysis results. The Panel was in agreement that there was potential for interaction between chemicals, but this did not preclude chlorpyrifos as a contributor. The Panel also noted that Rauh did perform an

analysis of the neurodevelopmental effects while controlling for diazinon and the mixture of diazinon and chlorpyrifos (Rauh personal communication to the Agency dated August 12, 2008, found in docket, EPA-HQ-OPP-2008-0274). In this analysis, the authors stated that the combination of chlorpyrifos and diazinon produced slightly more significant effects for MDI than were seen with chlorpyrifos alone. Diazinon alone did not produce significant changes in MDI. This interaction is critically important in interpreting the results.

3) More analysis controlling for multiple exposures and examining additive effects would help to elucidate the role of other organophosphates, but these analyses were not necessary for the panel to affirm that chlorpyrifos likely played a role in the outcomes that were observed. These results are important for AChE inhibition to be considered as a mode of action.

4) Whyatt and Rauh (Columbia University) in their comments to the Panel (September16, 2008) described how they separated the neurodevelopmental effects of chlorpyrifos exposure from those of diazinon exposure. Some panelists questioned whether the biological effects of these anticholinesterase insecticides could be separated.

5) The Agency's use of the word "likely" at the end of the paragraph needs to be considered carefully. The Agency should ensure that the use of the word is not based heavily on the Columbia study in the absence of intermediate markers of biologically effective dose and early effect that further link exposure and outcome and or a clearer idea of mode of action from experimental studies.

6) It is unclear whether the blood levels of the three AChE-inhibiting insecticides are highly correlated. If there is a lack of correlation, it is less likely that they would confound or be responsible for the observed relationships between chlorpyrifos and neurological outcomes. However, it would take a much larger and more detailed study to elucidate the more complex issues involving chemical mixtures (the possibility of synergism, antagonism, potentiation, activation, etc.) Some panelists suggested that chlorpyrifos (or chlorpyrifos oxon) may inhibit certain xenobiotic metabolizing CYP isoforms which may make some individuals more susceptible to exposure to other chemicals (e.g. other OPs, carbamates, pyrethroid insecticides, or other environmental contaminants). This is may be due to the resulting declined efficiency in CYP metabolism in response to these exposures when preceded by exposure to chlorpyrifos (Note: work by Ernest Hodgson's group at North Carolina State University was mentioned). Organophosphorothiate insecticides, including chlorpyrifos, act as "suicide substrates" leading to inhibition of CYP450 (DeMatteis1974; Halpert et al. 1980). Thus, exposure to these other compounds may also affect the metabolism of chlorpyrifos.

7) Other issues with the Columbia cohort study (Whyatt et al., 2004) were identified. The level of diazinon in the homes was 8 times higher than that of chlorpyrifos suggesting that the exposure level to diazinon was 8 times higher. Diazinon is less toxic than chlorpyrifos, and chlorpyrifos is more lipophilic than diazinon. With the difference in lipophilicity, it was not surprising to see more chlorpyrifos than diazinon detected in the

cord blood. There was an additional OP insecticide (methyl parathion) as well as 5 carbamates (including propoxur) present in the homes. These anticholinesterases could be interacting with each other.

8) The following are some clarifications provided by one Panelist on the material presented in Appendix D:

   a. The Agency stated that exposure is underestimated by use of blood levels. The Panelist agrees that this is true for peak exposures and a single blood sample would likely miss the peaks. However, because exposure is multi-media and multi-route, for most women in the Columbia cohort (as well as in the general population) single high insults are likely limited, and exposure may more likely be a series of contact events that are lower in magnitude over time via various routes and media. Thus, spot samples may be a useful way to estimate average exposure for women in the study since there is much lower within-home variability of pesticide levels as compared to between-home variability (Whyatt et al., 2007).

   b. Description of blood levels and underestimation of exposure for diazinon and propoxur on page 50 of the Agency's issue paper should be edited for clarity in the document. As it appears now, the document reads as though these pesticide levels would be more heavily underestimated as compared to chlorpyrifos levels in the same blood samples.

In conclusion, notwithstanding the fact that the three studies are not totally comparable, the Panel found that there were more similarities than discrepancies across them. When considered along with animal data, chlorpyrifos is likely associated with adverse neurodevelopmental outcomes. Although the Mt. Sinai and Berkeley cohorts are less specific than the Columbia study, they support the overall findings of the latter. The Panel supported the statement that exposures to all three AChE-inhibiting insecticides may act in combination to produce the observed effects. The Panel agreed that there may, in fact, be additive effects or effects generated by a mixture of the agents. Although the authors of the Columbia studies have attempted to isolate the effects that would be associated with chlorpyrifos, the Panel noted it is difficult to quantify the contribution of other neurotoxic compounds in such simultaneous exposures. It was also noted by one panelist that other OPs, as well as other chemicals, may play a role in neurotoxic effects in light of a recent study by Eskenazi et al. (2008). The Panel concluded that although a mixture of chemicals may produce the observed effects, this did not rule out chlorpyrifos as playing a role. However, the fact that mixtures were involved in the measurements of all three studies does limit the conclusions relative to the specific role of chlorpyrifos in observed outcomes.

The majority of the Panel agreed that although the exposures in the Columbia study occurred at levels below the U.S. EPA reference dose and likely underestimate true exposures, the results of this study are of concern regarding adverse effects on normal neurodevelopment of children exposed *in utero* to chlorpyrifos. This is particularly true in light of evidence demonstrating that low levels of exposure to toxicants once thought to have adverse neurodevelopmental effects only at high levels (i.e. lead, mercury, and PCBs) are now known to produce significant effects at

lower levels. Organophosphates are known neurologic poisons designed to kill insects. People exposed to high levels of OPs may suffer seizures, coma and death (Etzel, 2003). There was disagreement among the Panel as to what conclusions can be drawn from observed neurodevelopmental outcomes regarding the cholinesterase inhibition mechanism of action. Many other associations have been found between DAP, DMP, DEP and neurodevelopmental and neonatal outcomes that indicate a role for other organophosphates. This can be supported by a cholinesterase inhibition mechanism of action or perhaps some different mechanism. Some panel members believed more strongly that there was likely a different mechanism of action other than acetylcholinesterase inhibition mediating these neurodevelopmental effects.

5. Human Information Available for Risk Assessment:

Ultimately, the Agency will assess potential risk to humans from current exposures to chlorpyrifos. Thus, data in humans provide a valuable tool for considering human outcomes, metabolism, and dose response. Under this context, the Agency has considered the extent to which data in the epidemiology studies and the deliberate dosing studies can be used quantitatively in the chlorpyrifos risk assessment (See Issue Paper, Section 2.3).

a. The epidemiology studies provide important information about potential human outcomes related to the potential effects of OPs on the developing brain. Moreover, they provide data which supports the human relevance of outcomes observed in animal studies. However, at this time, they have not been proposed for use in directly deriving the PoDs or UFs.

Each of the cohorts has been exposed to chlorpyrifos to some extent. However, in addition to chlorpyrifos, each cohort has been exposed to multiple pesticides, including other OPs. Determining the quantitative contribution of chlorpyrifos to the reported outcomes separate from the other OPs is challenging. This determination would be highly uncertain given the current state of the science on the dose response relationships for mechanisms (other than AChE inhibition) leading to effects on the developing brain. As the science evolves in this area, the understanding of TK and TD factors which impact toxicity to the developing brain will improve as will the dose response information in animals. With this improved understanding, the Agency may, in the future, be able to better characterize the linkage between blood or urinary levels of chlorpyrifos and/or its metabolites with health outcomes. At this time, the Agency has used the reported levels of chlorpyrifos and its metabolites simply as markers of exposure without an attempt to estimate actual exposure or dose to the tissues. The Agency is aware of an effort by Drs. Dale Hattis and Robin Whyatt to develop a physiologically-based pharmacokinetic (PBPK) model which includes a placental compartment for assessing tissue dosimetry to the fetus and which accounts for intra-species TK variability. The investigators then plan to use that model to estimate a human PoD from the blood biomarker reported in Whyatt et al (2003). This work has only just begun and will likely take several years. *Please comment on the Agency's conclusion to use the epidemiological studies primarily for purposes of hazard characterization and not for dose response assessment. Please also comment on the scientific support for or against this preliminary conclusion.*

Panel Response

The Panel concurred with the Agency that there are key limitations to the three epidemiological studies and that they should be used primarily for hazard identification. The Panel disagreed on whether the current epidemiological data provide sufficient evidence to suggest that the uncertainty factor for the cholinesterase inhibition endpoint be changed to accommodate the possibility of neurodevelopmental effects from low-level *in utero* exposures of chlorpyrifos. The majority position was that current epidemiological data do not provide sufficient evidence to increase the uncertainty factor for the cholinesterase inhibition to accommodate the possibility of neurodevelopmental effects. However, some panel members felt strongly that the current epidemiological data do provide evidence to indicate that the margin of safety should be increased. The Panel recommends that the Agency conduct a full formal weight of evidence evaluation for causality of the reported associations between exposure to chlorpyrifos and neurodevelopmental outcomes in the existing epidemiological database.

One of the key limitations of the epidemiological studies is that the exposure data were collected at single time point and lack information on the long-term exposure level and duration. A second limitation is that the subjects in two of the cohort studies (conducted by the Mt. Sinai and University of California-Berkeley researchers) had multiple chemical exposures including multiple AChE-inhibiting insecticides (see discussion for previous question). The Columbia cohort study (Whyatt et al., 2002) also had multiple chemical exposures,for example, to the insecticides, DDT (detected at 68% of the samples) and chlordane (detected in 78% of the samples). One panel member added that it may be more difficult to use neurobehavioral endpoints in dose-response assessment as they are qualitative in nature. Another limitation is that neurodevelopmental changes are caused by multiple factors, precluding an accurate dose-response relationship. The Panel disagreed on whether the epidemiological data, and in particular the neurodevelopmental findings, provide sufficient data to support changes to the default uncertainty factor

The Panel stated that data from human studies, in general, are of great value for assessing risks to human health. Besides epidemiological studies and deliberate dosing studies potential sources for data on the effects of chlorpyrifos in humans may come from poison control information on accidental and intentional exposure (decreased since 2001 when indoor uses of chlorpyrifos were voluntarily cancelled) as well as from occupational exposure data from workers in manufacture, formulation, transportation, and application of chlorpyrifos (e.g., pest control operators, pilots, flagmen, pickers).

Confounders are unavoidable in epidemiological studies (see Panel response to Charge Question 4). Confounders regarding the exposure agent may include impurity, mixture (synergism/antagonism with other pesticides or agents), vehicle etc. Confounders regarding the exposure are most commonly related to dose, route, and duration of exposure. Confounders associated with the subjects enrolled in the study may include age, gender, health/disease status, personal environment, and genetic factors such as isoforms and transporters in key toxicological events. Within the chlorpyrifos epidemiological datasets, determining the quantitative contribution of chlorpyrifos in exposures to mixtures may be possible by accounting for differences in chemical potency, pharmacokinetics and possible chemical interactions. Further

resolution of dose-response relationships may also come from the on-going data analysis by the Columbia University study group and any other long-term studies with improved exposure information and more quantitative measure of chlorpyrifos toxicity.

The Panel recommended that the Agency conduct a full weight of evidence evaluation for the neurodevelopmental outcomes. This generally requires a much wider scope of review that encompasses all available data; not only data for the given chemical, but also data for chemicals of similar structure and activities, e.g., OPs. To increase transparency and defensibility of the conclusions concerning the potential role of chlorpyrifos in contributing to neurodevelopmental outcomes, the weight of evidence analysis should specifically address criteria for causality including consistency, and take into account the results not only of the studies considered here but others and the supporting biological data. Such an exercise requires explicit consideration of criteria such as strength, consistency, specificity related to chlorpyrifos or to its anti-cholinesterase effects common to OPs as a whole, dose-response, temporal concordance and biological plausibility in a framework analysis similar to that which is conducted currently for hypothesized modes of action. This allows comparative analysis across assessments of consistency of weight of evidence determinations. The weight of evidence analysis might increase confidence in this case and potentially identify additional relevant analyses to address uncertainties such as the role of other pesticides in the observed associations. To the extent possible, once a hazard is established, an array of dose-response data in both animals and humans in the context of their relative associated uncertainties may also be helpful, recognizing that the magnitude of these uncertainties for the dose-response may generally be greater for animal species, owing to interspecies differences. Outliers need to be excluded, and the relative magnitude of uncertainties explicitly weighted in considering the relative contribution of epidemiological versus other types of data.

The Panel agreed with the Agency that there were limitations in the three epidemiological studies that precluded them from being used to directly derive the PoD or the uncertainty factor.The Columbia University cohort study could be used to determine bounding values for the levels of chlorpyrifos that might cause a measurable effect. In a similar way, data from epidemiological studies can also be used in risk assessment. .The use of a PBPK model would enable estimation of an exposure dose metric for multiple sources of exposure, e.g., air, food, water.

For example, one Panel member estimated the blood concentrations of chlorpyrifos from the PBPK model provided by Dr. Timchalk (Battelle Center for Biological Monitoring and Modeling -Public comments dated Aug 28, 2008, submitted to docket EPA-HQ-OPP-2008-0274)) at the 50th, 75th, 95th percentile at the maximum of the California air concentrations of chlorpyrifos as stated in oral public comments provided by Dr. Sass of the Natural Resources Defense Council (NRDC and representing the Pesticide Action Network – North America (PANNA), September 16, 2008). Based on this analysis, the simulated chlopryrifos blood concentration was approximately $1x10^{-4}$ µmol/L, or 35 ng/L, when calculated using a peak air concentration of 1.34 ug/m^3. Some panel members raised concerns about this computation.[3] In

[3] One panel member provided some additional notes to clarify the vague concerns expressed at the SAP meeting. This information will be useful to the Agency. Review of public comments in the docket (EPA-HQ-OPP-2008-0274-0056,) indicated that the 24-hour average concentrations were below the adult RELs (recommended exposure levels). This finding raises a concern about the calculations made by one panel member. A lag time is needed to

this illustration, potential exposures to a blood concentration of 35 ng/L chlorpyrifos would be nearly 6 times greater in comparison with the measured chlorpyrifos blood concentration, of 6.17 ng/L reported in the Columbia University study. In the Columbia University study, levels of 6.17ng/L chlorpyrifos in blood were associated with neurobehaviornal effects. The Panel urged the Agency to more carefully analyze these current data to determine whether individuals exposed to potentially higher levels of chlorpyrifos in air would show signs of neurodevelopmental effects. In a similar fashion, data from the epidemiological studies could be used in risk assessment as bounding values to evaluate exposure standards, i.e., reference concentrations or doses. Based on the level of analysis that can be performed, Panel members suggested that the Agency might be able to use the cohort data in a more quantitative fashion as part of the risk assessment, such as in the aforementioned boundary setting exercise.

While the potential contribution of the chlorpyrifos PBPK model being developed was recognized, some panelists believed that the Agency should also pursue a simpler PBPK model specifically applicable to the chlorpyrifos data that would be available in a relatively short timeframe. Some panel members urged the Agency to not delay in studying whether the level of chlorpyrifos in air, as noted in the NRDC and PANNA comments, poses a concern for human health.

The Panel recommended the consideration of a fetal or placental compartment in a PBPK model should there be any indication from the paired maternal and cord blood data that chlorpyrifos and its active metabolite(s) are differentially distributed to the fetuses. In addition, the PBPK model can also simulate inter-individual (intraspecies) TK variations as well as inter-species differences. Furthermore, the Panel noted that additional work as a basis to consider more robustly interspecies differences (using central estimates) may be more fruitful over the shorter term.

b. Three deliberate dosing studies in adult (non-pregnant) humans are available which measure AChE activity and urinary levels of chlorpyrifos and/or its metabolites (See Appendix G). The Agency has determined that the deliberate dosing studies in adults are not appropriate for use in PoD or UF derivation in the current proposal. This determination is based on several factors.

- There are experimental laboratory animal data that indicate that the susceptibility of the developing nervous system to chlorpyrifos may be related to cholinergic and noncholinergic mechanisms. Findings in epidemiology studies in children support the animal studies. The human studies do not include the potentially susceptible populations being evaluated in the current effort, namely pregnant woman and children and thus do not consider toxicity endpoints other than AChE inhibition (and related clinical signs).

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reach a steady-state between air concentration of chlorpyrifos and blood levels, so that peak concentrations lasting one hour should not be used for calculating blood concentrations. A time-weighted average must be used instead, which would lead to lower blood levels.

- Nolan et al. (1982) and Griffin et al. (1999) only include a single dose group for a particular route (a study design issue previously criticized by the Human Studies Review Board (HSRB) with respect to other human studies).

- Griffin et al. (1999) report no changes in AChE inhibition and in Kisicki et al. (1999) changes were only seen in one person leading to the characterization of these studies as NOAEL only studies (a type of study not supported by the HSRB for use in risk assessment since absence of an effect (LOAEL) raises questions about whether the investigators were able to detect an effect or that it was possible given the study design).

However, the Agency has determined that the human studies do provide valuable information on correlating oral or dermal exposure with levels of chlorpyrifos and/or TCP in blood and urine. In addition, these studies also provide information on time course of absorption, metabolism, and excretion. Kisicki et al. (1999) also includes PON1 genotype information. Due to the availability of quality TK information, these studies have been used in the past by the Agency to aid in interpreting biomonitoring data. Specifically, results from Nolan et al. (1982) have been used previously by the Agency in estimating (i.e., back-calculating) chlorpyrifos exposure based on urinary levels of TCP. Nolan et al. (1982) has also been used to derive a dermal absorption factor in humans. If the Agency wishes to continue this use of the human studies to assist in characterizing and interpreting epidemiology and biomonitoring data, the studies will be brought to the Agency's HSRB for review of their scientific and ethical conduct. *To assist the Agency in preparing for this review by the HSRB, please comment on the clarity and completeness of the Agency's scientific analysis of the human studies. In particular, please focus on whether the Agency has identified the key scientific issues and whether other information or studies are available that should be considered in formulating the Agency's preliminary conclusion to use these studies for purposes of characterizing and interpreting the epidemiology and biomonitoring data and not for deriving PoDs or UFs.*

Panel Response

The Agency's issue paper clearly laid out EPA's scientific analysis of the human studies and identified desirable components that are missing in these human studies. One panel member noted that to enhance clarity for readers not intimately familiar with EPA procedures, information about human subjects and the conduct of the study should accompany the synopses of the studies provided to the Panel.

Overall, the Panel agreed that the human deliberate dosing studies contain scientifically useful information for risk assessment, but not for directly establishing PoD or uncertainty factors. The following major limitations of these data were noted by the Panel. Collectively, these limitations preclude their direct use for establishing PoDs.

- These studies do not include the potentially susceptible populations (e.g., women of childbearing age, pre- and post natal stages, teenagers and young adults before brain development is complete in the mid-20's).
- The single dose regimen does not provide information to evaluate dose-response relationship and span the range of environmental exposure levels.

- These studies do not provide information on any other toxicological endpoints besides cholinesterase inhibition.
- These studies do not include the inhalation route, a major route of exposure for workers and bystanders alike.

One panelist provided the following information to the Panel from his unpublished study conducted on a small cohort of children (n = 50) examining cholinesterase activity and PON1 genotype as a result of chlorpyrifos exposure (hand wash measurements for exposure). Two children with comparable exposures showed a 100-fold cholinesterase difference at the highest hand rinse concentrations, but different (slow versus fast) polymorphisms in the PON-1 genotype. On the other hand, two other children showed a 10-fold variation in cholinesterase activity even though they had the same PON-1 genotype. These studies used a single dose that may not reflect environmental concentrations and examined only dermal exposure. A significant portion of the observed differences in cholinesterase activity may be due to differences in inhalation or dietary exposures rather than dermal exposure (the route of exposure measured in this study). Given the small population size, use of two isolated comparisons, and the uncertainty associated with exposures, this study does not provide enough information to influence a choice of uncertainty factor. However, these unpublished results do confirm that genetic differences between individuals in a population may alter the exposure to toxic chlorpyrifos metabolites.

In principle, the Panel agreed with the Agency's scientific approach to derive a dermal absorption factor from the human studies. These human studies contain valuable scientific information especially if no other source of data is available. However, the current 3% absorption factor should be further refined by considering the different exposure scenarios. Taken together, these studies provide more robust database than what each single study can offer. They span a wide range of exposures, between 0.06 mg/kg (5 mg for 67-81 kg subjects in the Meuling et al. (2005) study) and 5 mg/kg (subjects B-F in the Nolan et al. (1982) study), different wash-off durations (as short as 4 hours in the Meuling et al. (2005) study), and using different vehicles.

The Panel recommended that if the Agency wishes to use these studies to derive dermal absorption factors for use in risk assessment, then they should adjust for any underestimation using the apparent inverse relationship between dermal absorption and the concentration of exposure (see Nolan et al., 1982 and Meuling et al., 2005.) The Panel also noted that when deriving the dermal absorption from each study, the estimated oral absorption in the same study should be used as a reference point instead of the 100% oral absorption that was assumed in extrapolating data from oral and dermal routes. When necessary, material balance and elimination kinetics from each study should also be accounted for. Other limitations of these studies should be considered as uncertainties, e.g., exposure only on forearms and shorter duration of skin contact than the anticipated human exposures. The Panel was familiar with how the 3% dermal absorption factor used by the Agency (see EPA's 2000 chlorpyrifos risk assessment) was derived based on the Nolan et al. (1982) study. The Agency compared this factor to the 0.03 ratio between the oral LOAEL of 0.3 mg/kg/day from the rat DNT study to the 21-day dermal LOAEL of 10 mg/kg/day in rats. Because it is not likely that the dermal absorption factor derived in humans would closely coincide with the values for rats, and given

that the comparison of toxicity at the LOAELs is crude, the support from this comparison would not be crucial.

The Panel agreed with the Agency scientific analysis that these deliberate dosing studies cannot be used to directly establish a PoD or UFs. The Panel indicated that these data might be used as bounding levels similar to what was suggested by the Panel concerning the data from the epidemiological studies (see Panel response to Question 5(a)).

The Panel appreciated the Agency's scientific analysis to compare the blood levels in the deliberate dosing and epidemiological studies, and considered it critically important to maximally use the information from these studies. These studies could be used for purposes of interpreting (at least crudely) the nature of dose-response in the epidemiological data and as a basis to "bound" the reference doses/concentrations. The Panel encouraged the Agency to consider the use of a PBPK model to widen the application of these bounding data for current or potential human exposures and for the final reference dose or reference concentrations. In addition, these human study data may contribute to an array of the dose-response data in both animals and humans mentioned under question 5a, but in the context of their relative associated uncertainties.

6. Points of Departure (PoD) for Risk Assessment (Issue Paper, Section 2.5):

a. Based on the results of the extensive literature review, the Agency has proposed updated PoDs derived from the laboratory animal studies for extrapolating human risk. The Agency has posed three options for the PoDs for chlorpyrifos in its issue paper. When the Agency derives PoDs for assessing the risk from exposures to pesticides, it needs to consider all relevant routes (oral, dermal, inhalation), durations (ranging from acute to chronic), and all exposed populations (including adult, pregnant women of child bearing age and children).

The first option proposes to use the PoDs which were based on rat RBC and plasma cholinesterase inhibition in the 2000 risk assessment for acute oral exposures and blood AChE for chronic oral exposures. The 2000 risk assessment included a weight of the evidence discussion primarily on adult rat and dog AChE guideline studies and adult data from Zheng et al. (2000). This option would involve application of the no-observed-adverse-effect-levels (NOAELS) for blood AChE inhibition from route specific studies (oral, dermal, inhalation) in rats or dogs to all populations. The acute oral PoD would be 0.5 mg/kg/day and the repeated oral PoD would be 0.03 mg/kg/day. The dermal and inhalation NOAELs would be 5 and 0.1 mg/kg/day, respectively.

The second option proposes to use a value of 0.1 mg/kg/day derived from multiple studies and lifestages. This proposed PoD would be applied to all populations and all durations. The proposed value of 0.1 mg/kg/day was derived using benchmark dose estimates from brain and RBC AChE in young pups (PND1 and 12) following acute dosing and from peripheral (heart) AChE following repeated gestational studies with dams. As such, multiple lifestages are considered in the proposed PoD: pregnant dams, PND1 pups, and PND12 pups. Furthermore, the proposed value is 3 to 10 fold lower than causing effects on the developing brain reported in other laboratory animal studies and thus is expected to be protective for those effects.

The third option is a blend of options 1 and 2. This option proposes to use a value of 0.1 mg/kg/day derived from the acute post-natal rat brain and RBC AChE data for all populations but only for the acute duration for oral exposures. Exposure scenarios involving repeated exposures would use the PoD of 0.03 mg/kg/day from the 2000 risk assessment for oral exposures. The value of 0.03 mg/kg/day was derived for the 2000 risk assessment based on blood AChE from multiple adult rat and dog studies. The lower PoD for repeated exposures in option 3 is proposed to account for potential accumulation of toxicity which can occur following repeating doses of chlorpyrifos in adult studies. *Please comment on the strengths and weaknesses of each proposed approach. Is there another option or a variation of one of the three options that the Agency should consider?*

Panel Response

General comments:
The principal focus of the questions from the Agency on PoD relates to whether PoD used in the 2000 chlorpyrifos risk assessment will be altered given more recent data on neurodevelopmental effects associated with early exposure to chlorpyrifos. Recommendations here are predicated on some general principles in selecting PoDs for risk assessments, including making maximal use of route specific data as a basis to minimize uncertainty, to rely to the extent possible on benchmark doses (BMDs) versus no- or lowest-observed-(adverse)-effect levels (NOAEL or LOAEL) and to take into consideration the sensitivity of all life stages.

Members of the panel also noted that it was difficult to separate fully the discussion of points of departure from that of uncertainty factors so that the content here has relevance to the responses to Question 7 and vice versa (i.e., the adequacy of proposed uncertainty factors is necessarily related to the nature of the point of departure). As a result, comment is also included herein on residual uncertainty associated with proposed or preferred points of departure including that related particularly to potential lack of adequate consideration of all life stages.

The Panel agreed with the Agency's decision not to use the human deliberate dosing studies to establish PoDs due to their limitations, in particular their lack of account of potentially sensitive life stages.

Specific Comments:
There was consensus that of the three options proposed by the Agency, Option 3 is preferred, based on the available data. This is predicated principally on the basis that the proposed PoD for acute exposure takes into account all life stages, is based on benchmark doses which offer an advantage over the NOAEL or LOAEL, and represents the results of several studies, for which results converge around the same value. Based on available data, most of Panel members (a few disagreed) stated that the PoD presented in Option 3 is also believed to be protective for effects on the developing brain, although it is based on cholinergic effects. However, this conclusion is highly uncertain, given the lack of information on the mode of induction of the observed behavioral effects and in light of evidence from *in vivo* and *in vitro* studies that non-cholinergic mode of action(s) are likely involved in the adverse developmental neurotoxicity (DNT) and

behavior endpoints. The Agency is encouraged, therefore, to explicitly address this uncertainty in their additional deliberations in derivation of the reference dose/concentrations.

The Panel suggested that if the Agency wanted to test whether the cholinergic pathways are an appropriate endpoint for the PoD then an appropriate temporal dose-response study using the most sensitive administration method and the most sensitive life-stage comparing DNT/behavior with cholinesterase inhibition would be needed. This would test the hypothesis that cholinergic pathways are an appropriate endpoint for the PoD, even though such a study would likely not elucidate specific non-cholinergic mode of action(s) for DNT/behavior effects. Relevant design would need to take into account differing sensitivity in various species to detect effects on the developing brain.

The neurobehavioral developmental effects seen in the three epidemiological studies supported the retention in Option 3 of the lower PoD for repeated exposures used in EPA's 2000 chlorpyrifos risk assessment to account for potential cumulative effects. For repeated exposures, PoD of 0.03 mg/kg/day is supported by a $BMDL_{10}$ of 0.03 mg/kg/day for RBC AChE inhibition in pregnant dams (see Agency issue paper, p. 18, Table 2). The Panel also recommended that the Agency additionally investigate developing appropriate benchmark doses (BMDs) for the chronic PoD determination.

More detailed comments:

Option 1: Option 1 uses the rat RBC and plasma ChE inhibition in the Agency's 2000 risk assessment (Table 1 of the Agency's issue paper), weight of evidence (WOE) approach based primarily on adult rat and dog AChE guideline studies, NOAELs for various routes and durations of exposure with an acute oral dose of 0.5 mg/kg/day and a repeated oral dose of 0.03 mg/kg/day.

Option 1 is the status quo used in the Agency's 2000 chlorpyrifos risk assessment based on studies conducted solely in adults (blood AChE for acute and chronic oral exposures). The additional data on neurodevelopmental effects would have no impact at this time. As per other options, it has the advantage that it avoids uncertainties associated with interroute extrapolation. However, it is based on no-or lowest-observed-advese-effect-levels rather than benchmark doses; moreover, the dermal and inhalation NOAELs were determined in mature animals and did not include protection for pre- and post-natal sensitivity.

Option 2: Option 2 uses studies that provide data amenable to benchmark dose (BMD) modeling; RBC AChE studies in repeated gestational studies in dams (heart) and acute post-natal pups (brain and RBC AChE) provide BMDs (BMD and $BMDL_{10}$) in the same range of 0.06 – 0.12 mg/kg/day. The Agency proposes to use the weight of evidence approach, a PoD of 0.1 mg/kg/day for oral exposure, all age groups and all durations.

For Option 2, a value of 0.1 mg/kg/day is being proposed for all populations and durations. It is based on benchmark doses/concentrations which offer some advantage over NOAELs/LOAELs though it needs to be recognized that these advantages can be small in relation to relative uncertainties associated with interspecies and intraspecies differences. It does draw from

multiple studies covering several life stages (brain and RBC) AChE in young pups following acute dosing – PNDs 1, 7 and 12 and peripheral AChE from repeated gestational studies with dams; also pups). The Panel was reassured that the values for several studies fall within the same range and the approach offers considerable advantage in drawing maximally on the available data in animals at several life stages. Some panel members stated that 0.3 mg/kg/day was protective because it is 3- to 10-times lower than levels causing effects on the developing brain. The lowest dose tested for learning/memory was 0.3 mg/kg/day so proposed PoD was 3-fold lower and 10-fold lower than lowest dose used in many gestational and post-natal studies which evaluated toxicities other than AChE inhibition. Nevertheless, the degree of protectiveness is uncertain because the dose-response relationship cannot be obtained from these studies reporting substantial effects at 0.3 to 1.0 mg/kg/day. Another consideration is that the 0.1 mg/kg/day dose does not take into account potential for cumulative effects following repeated exposure.

Option 3: Option 3 separates the PoD for acute and repeated exposures. A PoD of 0.1 mg/kg/day was used for acute and short term exposures. For chronic dietary exposure, a value of 0.03 mg/kg/day was used. The value for repeated exposure is based on NOAELs and LOAELs for plasma and RBC ChE inhibition in 5 studies. This option is also supported by the BMD in pregnant dams.

These PoDs (0.1 and 0.03 mg/kg/day) are 3 and 10 times, respectively, lower than the lowest dose tested across all neurodevelopmental studies (0.3 mg/kg/day). The latter dose was administered to dams through gestation and even several postnatal days without observing behavioral effects at any time in the offspring exposed to this dose level (see Agency issue paper, p. 19). The PoDs for acute and repeated exposures might be considered 3-fold and 10-fold higher than the uncertainty factor (TD), respectively, for neurodevelopmental effects. Option 3, is essentially Option 2, but introduces a lower value for repeated exposures from the 2000 assessment to account for potential accumulation of toxicity which can occur following repeated doses. This option was preferred by the Panel, on the basis of potential for different or perhaps multiple actions of chlorpyrifos at repeated low level exposures and results of the recent epidemiological studies. Most of panel members (a few disagreed) stated that this option was protective of effects on the developing brain, although it is based solely on cholinergic effects. However, this conclusion has associated uncertainties; the lack of information on the mode of action in inducing the observed behavioral effects and evidence from *in vivo* and *in vitro* studies that show non-cholinergic modes of action are likely to be involved in the adverse developmental neurotoxicity and behavior endpoints. The Panel encourages EPA to explicitly address these uncertainties when deriving the reference dose/concentrations.

b. Route specific data are preferred because such data accounts for potential differences in absorption, distribution, or metabolism. In the case of chlorpyrifos, dermal and inhalation studies are available which identify NOAELs for these routes in adult rats. With respect to inhalation exposure, there are two nose only studies with vapor chlorpyrifos which provides a NOAEL of 287 ug/m3 or 20 ppb (0.1 mg/kg/day). Similarly, there are two dermal studies which together provide a dermal NOAEL in adult rats of 5 mg/kg/day. These studies do not include pregnant dams, fetuses or post-natal pups and therefore do not consider potentially susceptible populations. In the absence of data in these groups, the Agency will continue to use route specific studies, as appropriate. An alternative for dermal exposure is to use an oral Pod derived

from susceptible populations (as discussed above) with a dermal absorption factor. Specifically, the Agency could use a dermal absorption of 3% from human subjects (Nolan et al., 1982). *Please comment on the strengths and weaknesses associated with use of the adult dermal and inhalation studies in the chlorpyrifos human health risk assessment. The Agency also requests the SAP to provide suggestions on potential toxicity and/or toxic kinetic studies (if any) in pregnant dams, fetuses, and/or post-natal pups which could be conducted to better inform the dermal and inhalation risk assessments.*

Panel Response

The Panel was in general agreement with the Agency's proposed option to use the dermal and inhalation studies as a basis for development of the points of departure for these routes for repeated exposures, as per the 2000 chlorpyrifos risk assessment given the uncertainty associated with route to route extrapolations based on available data, though residual uncertainty concerning sensitive life stages would need to be taken into account. The Panel also noted that the proposed oral, inhalation and dermal PODs are roughly equivalent (0.1 mg/kg/day), taking into account estimated absorption from the Agency's 2000 chlorpyrifos risk assessment based on the Nolan et al. (1982) study. However, proper adjustment of the dermal absorption factor for use in oral to dermal extrapolation is needed when the in-study oral absorption factor is less than 100%. Other considerations for deriving and using the dermal absorption factor in response to question 5(b). Inhalation Pod of 0.1 mg/kg/day should also be adjusted for repeated exposures based on the 5 days per week dosing regimen.

Also, rather than relying on the potentially proposed value of 3% for dermal exposure derived in the deliberate dosing study in humans by Nolan et al. (1982), the Agency is encouraged to review the entire database from all doses and all studies to obtain quantitative understanding of age sensitivity specific to the endpoint of choice, as a basis to apply it across all routes of exposure consistently. Physiologically based pharmacokinetic modeling may be helpful in this context. While the Agency is pursuing development of a PBPK model that includes the fetal compartment, the Panel also encourages consideration of simpler models which might additionally inform comparison of age- and route sensitivity in the near term future. In relation to dermal exposure, the Panel suggested a more relevant species, e.g., minifies, may be helpful.

Detailed Comments:

Given the magnitude of the uncertainties to extrapolate between routes, use of route specific data for inhalation and dermal exposure as a basis for points of departure was supported. However, the residual uncertainty for the lack of relevant data in pregnant dams, fetuses or post-natal pups exposed by these routes needs to be additionally addressed.

Inherent in calculating the absorbed dose from the inhalation NOAEL is the recognition that the dose level should be somewhat comparable between routes for the same systemic endpoint, although rout-specific pharmacokinetic differences can be a significant factor. Some comparisons between dermal or inhalation NOAELs and the oral PoD are presented below.

Dermal NOAEL of 5 mg/kg

Using the Agency's suggested 3% dermal absorption factor derived from the deliberate dosing study in humans of Nolan et al (1982), the absorbed dose for the dermal NOAEL is 0.15 mg/kg/day, not significantly different from the 0.1 mg/kg/day value in Option 2. However, because the extent of oral human absorption estimated from the same study (i.e., Nolan et al., 1982) is 73%, the oral NOAEL of 0.1 mg/kg/day would need to be adjusted by 73% of its original value for this comparison. Thus, the acute dermal NOAEL of 5 mg/kg is approximately 2-fold higher than the oral BMD.

The Panel commented that it is important to recognize that when used in human risk assessment, the magnitude of the absorption factor is dependent on the dermal exposure conditions. For example, in the Nolan et al (1982) study, the absorption factor was 3% at 0.5 mg/kg, but only 1% at 5 mg/kg. The Panel noted that it is important to consider other factors that could affect dermal absorption, e.g., variation of rate of penetration on different skin surfaces, moisture on the skin under the cover of clothing, contact duration before wash off.

Inhalation NOAEL of 0.1 mg/kg/day

According to the Agency's Issue Paper, the NOAEL of 20 ppb from the two inhalation studies in rats resulted in an absorbed dose of 0.1 mg/kg/day, the same value as in Option 2. The value, as calculated, is therefore applicable to acute exposures. For intermediate and chronic inhalation exposure, the absorbed dose of 0.1 mg/kg/day should be adjusted down by a factor of 5/7 to account for the 5 days/week dosing regimen. This is slightly lower than the 0.1 mg/kg/day value as proposed in Option 2.

Overall, the Panel encourages the Agency to review the entire database from all doses and all studies (not just the Nolan et al. (1982) study) to obtain quantitative understanding of age sensitivity specific to the endpoint of choice and apply it across all routes of exposure consistently. Physiologically-based pharmacokinetic modeling may be helpful in this context. While the Agency is pursuing development of a PBPK model that includes the fetal compartment, the Panel also encourages consideration of simpler models which might additionally inform comparison of age- and route sensitivity in the near term future.

7. Extrapolation/Uncertainty Factors (Issue Paper, Section 2.6, Appendix E):

In risk assessment, once PoDs are selected, extrapolation from animals to humans (inter-species) and within human variability is performed. Historically, the Agency has used default 10-fold factors to account for inter- and intra-species extrapolation. More recently, emphasis on the derivation of extrapolation factors from TK and toxicodynamic (TD) data instead of default factors has increased. With the intent of improving the scientific basis for the chlorpyrifos risk assessment, in this issue paper the Agency has considered the availability of current PBPK models, TK, and TD data for chlorpyrifos to use in animal to human and within human extrapolations. Overall, the available PBPK models, although well-developed and supported for non-pregnant adults, do not include calculations for dose during pregnancy (e.g., no placental compartment) and for young children less than 5 years old and thus cannot be used in a quantitative manner for this effort. As such, the Agency has used the 2005 IPCS guidance on Chemical-Specific Adjustment Factors to evaluate available TK and TD data in animals and

humans and to determine the extent to which such data support data-derived or chemical-specific extrapolation factors.

a. Inter-species and Intra-species Toxicodynamic Extrapolation: The Agency has preliminarily concluded that with regard to TD characteristics, due to the likelihood of several possible modes of action of neurodevelopmental toxicity of chlorpyrifos and lack of identifiable and quantifiable key events for MOAs not related to AChE inhibition, the Agency cannot confidently refine the TD component of the animal to human and within human variability factors (i.e., UF_{AD} and UF_{HD}). *Please comment on the scientific support for or against the use of default factors of inter- and intra-species TD extrapolation.*

Panel Response

In response to the question of whether the Agency can refine the toxicodynamic (TD) component with respect to uncertainty of the animal to human and within-human variability, the Panel was in agreement with the Agency that there is not sufficient information to confidently refine the TD component of the animal to human and within human variability factors (i.e., UF_{AD} and UF_{HD}). The Panel agreed that neurodevelopmental effects in the fetus and neonate are important endpoints for chlorpyrifos toxicity, but that there are several possible modes of action of neurodevelopmental toxicity of chlorpyrifos and there is a clear lack of identifiable key events for mode of actions not related to AChE inhibition. One panel member commented that the Agency should also consider AChE inhibition rather than behavioral outcomes, 0.03 mg/kg/day versus 0.3 mg/kg/day, respectively, because of its higher level of sensitivity. Overall, the lack of identified mode of action, lack of correlation of acetylcholinesterase inhibition with possible non-cholinergic mechanisms and developmental outcomes, taken together, do not support a data-derived inter-species and intra-species TD extrapolation. All panel members concurred that the application of uncertainty factors is linked to the effect and dose at the PoD and the issues related to PoD described in Question 6 are relevant here.

Based on consensus around sensitivity discussed in Questions 1 and 2, panel members agreed that animal and human sensitivities are greater for fetuses and neonates, although the current data do not allow for determination of the magnitude of the sensitivity. Until this is known, the Panel recommended application of default factors for the TD uncertainty extrapolation. A few members of the panel suggested that the Agency search the open literature for specific information and perhaps seek additional data regarding xenobiotic chemical effects on the developing nervous system. This information might show variations in response among developing brains due to genetics, time of exposure, pre-existing health status and other factors and support the adequacy of a 3-fold uncertainty factor (UF) for intra-species variation for these endpoints.

In conclusion, until a mode of action is identified and supported (i.e., primary target other than AChE activity) for neurodevelopmental toxicity is identifiable and consistently documented *in vivo*, the default uncertainty factors for the toxicodynamic component should be used. That is, 3-fold and 10-fold, for acute and chronic exposures, respectively, for neurodevelopmental effects.

b. Inter-species and Intra-species Toxicokinetic Extrapolation: As discussed in detail in Appendix E, the Agency evaluated the extent to which data on carboxylesterases, P450s, and paraoxonase (PON1, or A-esterase) support development of DDEFs of inter- and intra- TK extrapolation (i.e., UF_{AK} and UF_{HK}). Based on differences in rat and humans with regard to maturation of metabolic processes, there are uncertainties surrounding appropriate metabolic parameters for animal to human extrapolation of juveniles. This uncertainty in combination with limited data precludes the development of a DDEF for inter-species TK extrapolation (i.e., UF_{AK}). Thus, the Agency proposes to apply the default 3X for UF_{AK}. Data on carboxylesterases are not sufficiently robust for intra-species TK extrapolation (i.e., UF_{HK}). Data on P450s are complicated by multiple enzymes each with its own maturation profile. Others have evaluated the P450 literature for use in derivation of child specific UFs with poor success (Ginsberg et al., 2004a). *Please comment on the scientific support for or against the use of default factors of inter-species TK extrapolation. Please further comment on the Agency's preliminary conclusions on the utility of carboxylesterase and P450 data to refine the intra-species extrapolation factor.*

Panel Response

The Panel concurred with the Agency that there is scientific support to use a default factor for inter-species TK extrapolation. The Panel agreed with EPA's decision to apply the default UF_{AK}. The Panel unanimously encouraged the Agency to continue to consider the importance of all the enzymes in chlorpyrifos' metabolic pathway; the P450s, the carboxylesterases and PON1 as chlorpyrifos' toxicity appears to be dependent on the active metabolite, chlorpyrifos-oxon. The PON1 activity towards the chlorpyrifos-oxon, i.e., chlorpyrifos-oxonase is involved in the catalytic activity of the enzyme, but displays genetic polymorphism. There are important differences in the rates of hydrolysis (enzyme activity) across genotypes (192RR > 192QR > 192QQ), with PON1 R192 allele hydrolyzing chlorpyrifos with a higher catalytic efficiency than PON1 Q192 allele (Furlong, 2007). However, PON1 activity and polymorphism at position 192 (referred to as PON1 status) is not the only determinant of chlorpyrifos toxicity, since other metabolic pathways may modulate potential deficits in detoxication capacity. Thus, at least theoretically, the level of chlorpyrifos-oxon present in serum will protect against the circulating active metabolite. However, at low dose exposures, most active metabolites generated in the liver are also detoxified immediately in the liver, either catalytically (PON1 and CYP450) or stoichiometrically (carboxylesterase and butyrylcholinesterase, BChE)). One panel member commented that research by Sogorb et al. (2008) indicated that even if a small fraction of chlorpyrifos-oxon leaks from the liver without being detoxified, it is rapidly bound and inactivated by serum BChE and also by albumin, demonstrating the hydrolytic activities of BChE against chlorpyrifos oxons at concentrations lower than 0.5 μM (Sogorb et al, 2008). Mattsson et al. (2000) detected chlorpyrifos-oxon in blood of fetuses at a concentration of 1 ng/g (that equals to 0.003 μM) only after the administration of high doses of chlorpyrifos (5mg/kg/day) by gavage to dams at gestational day 20. Such a low concentration of chlorpyrifos-oxon can be efficiently detoxified by serum albumin regardless of PON1. On the other hand, no chlorpyrifos-oxon was detected in blood samples from human volunteers exposed to single oral doses of chlorpyrifos ranging from 0 to 2 mg/kg (Kisicki et al., 1999). Even in the case that some chlorpyrifos-oxon escapes from binding to blood proteins or enzymes, and from being hydrolyzed by PON1 in the blood; the possibility that it reaches the brain is scarce since chlorpyrifos-oxon is a highly reactive metabolite and undergoes spontaneous hydrolysis (its half-

life is less than one minute in blood, see Brzak et al., 1998), so there is not enough time to complete that journey intact. Thus, PON1 probably fails to play a relevant role at low environmental concentrations (nM to low μM). The maximum daily intake of chlorpyrifos is 0.11 μg/kg/day for infants and 0.24 μg/kg/day for toddlers (see review by Eaton et al., 2008; Table 21). At middle and higher concentrations, chlorpyrifos-oxon may be formed in the brain because of the low desulfuration activity of brain microsomes and mitochondria (Chambers and Chambers, 1989). However, the brain has no PON1 activity and, therefore, the oxon produced "*in situ*" interacts with its target molecules unless it undergoes stoichiometric binding to BChE or other proteins acting as "scavengers".

Lassiter at al. (1998) demonstrated that maternal blood chlorpyrifos-oxonase activity is variable before birth, and the placenta has about 20% the activity of the liver, which is consistent during late pregnancy. Fetal liver exhibited minimal activity during gestation, but increased after delivery. Mortensen et al. (1996) showed that maternal and fetal brains had no detectable chlorpyrifos-oxonase activity (Mortensen et al, 1996). Although, the Km of chlorpyrifos-oxonase activity is high (Km=210-380 μM) , chlorpyrifos-oxonase in plasma and liver from adult animals has be shown to be capable of hydrolyzing physiologically relevant concentrations of chlorpyrifos-oxon (nM to low μM) whereas the young animal has, in turn, less capacity to detoxify such concentrations of chlorpyrifos-oxon via chlorpyrifos-oxonase (Mortensen et al., 1996).

One panel member pointed out that the lack of tissue specific data for both species under similar dose and timing considerations e.g., liver, is a major gap in the ability to extrapolate from animal data to humans. Others pointed out that there are human data on human liver carboxylesterases during maturation, but these data were not considered "sufficiently robust" to be useful in TK extrapolations.

c. Intra-species Toxicokinetic Extrapolation (Within Human Variability): There are extensive data on PON1 from many populations worldwide and for different age groups. Using these data, the Agency has performed a preliminary analysis for within TK human variability for PON1 activity. These calculations were done in a manner consistent with the IPSC CSAF guidance. The calculations show that the largest variability in PON1 activity is between newborns and their mothers and is thus likely related to age-dependant maturation.

There is some debate as to the extent to which PON1 status plays a role in toxicity at low environmental concentrations. Some have suggested that significant amount of OP (active oxons, not the parent components) must be present in the blood or brain for PON1 activity to affect toxicity based on generally low affinity (Km, 0.1-10 mM). Others believe that PON1 status is a key determinant in chlorpyrifos toxicity. The Agency has evaluated the available *in vivo* and *in vitro* data from animals and humans relevant to this issue. The Agency has preliminarily concluded that the available data suggest that PON1 status can not be ruled out as a determinant in chlorpyrifos toxicity, particularly for the fetus or young child. However, uncertainties remain, particularly regarding the degree to which other metabolic pathways modulate potential deficits in detoxication capacity. *Please comment on the science which does and does not support PON1 status as a determinant in toxicity at low environmental concentrations.*

Panel Response

The Panel unanimously concurred with the Agency, based on the data, that PON1 status cannot be ruled out as a determinant in chlorpyrifos toxicity, particularly for the fetus or young child. Several panel members stated that actual human exposures vary between bystander and applicators (and everyone in-between) and it is difficult to define what exactly a low level of exposure is for humans. Some in the Panel suggested that the PON1 dataset could be used to address data uncertainty. However, many in the Panel cautioned that these data should not be used out of context until the rate limiting step is identified based on a PBPK model. Such a model does not yet exist, but is under development. These panelists believed that the use of the PON1 data set without such information would be a misuse of the IPCS guidance for determining a CSAF (chemical-specific adjustment factor).

Several members of the Panel encouraged the Agency to obtain an independent peer review for the Timchalk PBPK model in order to assess the overall impact of PON1 relative to the toxicity of a range of CPF, including what may be the choice of dose metric for such comparison, e.g., BChE inhibition or blood or tissue level chlorpyrifos, chlorpyrifos-oxon, or TCP.

One panel member reminded the panel that there have been no direct experiments looking at what we might consider to be low dose exposures in animals in reference to the involvement of PON1. This panel member discussed two published model systems that might be of use to answer this question: the PON1 knockout (ko) and the PON1-Q192 humanized mice. In the PON1 ko model if there any effects of PON1 at low dose exposures then the panelist suggested that these effects would be manifested in this model. The Shih et al. (1998) paper shows that the presence of PON1 prevents the inhibition of brain acetylcholinesterase at a dose of chlorpyrifos of 300 mg/kg (see discussion in Appendix A (Metabolism), p. 26, Figure 8, Panel E of the figure reprinted from Shih et al., 1998). However, the magnitude of the difference between the wild type and the PON1 ko mice is on further reflection not that great. By this panel member's calculations the loss of acetylcholinesterase activity is ~60% in the ko mice and 40% in wt mice. This would then suggest that at lower chlorpyrifos exposures and in people with varying levels of PON1 activity (and not a complete lack of activity) there might be very little effect.

In the second model system, the PON1 humanized mouse model, if there are higher chlorpyrifos-oxon doses then there would be a significant difference in the inhibition of brain acetylcholinesterase in mice with either the R192 or Q192 polymorphism (see Appendix E, figure 4, p21). At low doses, it may not be so clear, particularly as there are no data for the R192 mice at the lowest dose used. The question then remains whether this results in a functional effect that can be identified. The original paper by Cole et al. (2005) also presents morbidity data in the same mice following these chlorpyrifos-oxon doses (Panel C of the same figure). Again there would appear to be missing data (namely there does not appear to be any data for the R192 mice below 1.5 mg/kg. However at 1.5 mg/kg there is no morbidity in the R-192 mice and with the Q192 there is no morbidity at doses below 1.0 mg/kg. Again this would suggest at "low" doses there might not be significant differences between the R/Q forms in preventing chlorpyrifos-oxon induced toxicity. This panel member concluded that further data are needed

but that current evidence is suggestive that PON1 is not a determinant of toxicity at low environmental concentrations.

d. The Agency's PON1 calculations have focused on the PON-192Q/R polymorphism based largely on the extensive data available. No calculations have so far been performed on other genotypes. *Please comment on scientific support for or against focusing on the PON-192Q/R polymorphism.*

Panel Response

The Panel concluded that the use of the PON192Q/R polymorphism in the Agency's calculations for PON1 intra-human variability is appropriate. This conclusion was based on the available data (Furlong et al., 2005; Brophy et al., 2001). However, the analysis of the individual contribution of each polymorphism in the promoter region on serum paraoxonase activity/levels is complicated because of the pronounced linkage disequilibrium between the promoter and the coding region polymorphisms (Draganov and La Du, 2004). Thus, it cannot be ruled out that the variation observed for one of the promoter polymorphisms (PON1 C-108T) may be in part due to linkage disequilibrium with the PON1 Q192R polymorphism (Brophy et al., 2001; Sirivarasaia et al., 2007). Other studies have actually excluded a significant effect of this polymorphism on serum PON1 (Phuntuwate et al., 2005).

The 2005 IPCS CSAF guidelines state that the sensitive subgroup should be evaluated as a distinct population (bi-modal distribution). The data from Holland et al. (2006) on mothers and infants are the two important sensitive subgroups (infants and those with the Q/R polymorphism). Intra-individual variability could be underestimated because this population is of similar ethnic descent (i.e., Latina mothers and infants, primarily of Mexican descent).

In the case of PON1, the sensitive group is the neonates with the QQ genotype. They should be (and are) compared back to the mothers of the QR genotype. If data from other studies exist (could be done now for adults with the data from Brzak et al. (1998), it would be appropriate to combine geometric means and geometric standard deviations by a weighted average approach. This would provide a more diverse group to be considered as the reference group (i.e. QR genotype, Latina mothers, white women and white men).

Several panel members made a strong recommendation that the Agency gather and analyze the data (*in vivo & in vitro*) from animal and human enzyme kinetics to look more closely at the PON-192Q/R polymorphism. Once a PBPK model is developed then the impact of activating and deactivating pathways and potentially rate-limiting components will be identified and information regarding PON1 Q/R polymorphism could be put into context. Such information, if attainable, might be used to modify the Agency's proposed approach.

e. The Agency's calculations conducted on PON1 activity follow the 2005 IPCS CSAF guidance for developing intra-species extrapolation factors for TK. The preliminary analysis suggests that within human variability is larger than the default 3X when newborns and adults are considered together. Specifically a value of 12X has been calculated for chlorpyrifos-oxonase activity. The Agency has proposed two options for these calculations: 1) use the value calculated

for chlorpyrifos-oxonase derived from newborn and mother values in Holland et al. (2006) and 2) use the default factor of 3X. *Please comment on the strengths and weaknesses of each proposed approach. Please include comments on the statistical approach used in the analysis as a component of your response.*

Panel Response

The panel reviewed the two approaches and the majority of the panel concluded that the currently available data indicate that the UF_{HK} should be the default value of 3-fold when newborns and adults are considered together. On the other hand, a few panel members concluded that the currently available data indicate that the overall UF (UF_{HK} x UF_{HD}) should be greater than 10 based on PON1 data (not other enzymes involved in the metabolism of chlorpyrifos) and the potential for developmental neurotoxicity of chlorpyrifos as noted in the epidemiological studies (Rauh et al). These members preferred the UF_{HK} of 12-fold, rather than the default of 3-fold, because these were the only two choices proposed. However, none of the panel members endorsed the CSAF approach used by the Agency to identify the factor of "12-fold" calculated based on chlorpyrifos-oxonase and encouraged the Agency to pursue other approaches based on the mode of action (see 2005 IPCS CSAF guidelines). Many panel members did not endorse the 12-fold uncertainty factor because it focused strictly on PON1 as the focal point of toxicity, i.e., focused on only one enzyme out of a very complex system. Similarly, one panel member stated that use of the 12-fold uncertainty factor would be a complete misuse of the 2005 IPCS CSAF guidelines in which there is a clear need to define uncertainty factors based on the endpoint linked to the mode of action, neither of which is defined in this case.

Members of the panel expressed concern about lack of data for non-plasma enzymes, no biological reason or data given support the notion that maturational differences in one tissue relate to another. The data on the relative contributions of P450, carboxylesterases and butyrylcholinesterase to the ultimate concentration of oxon in the target tissue(s) is insufficient to derive a data-derived uncertainty factor, as EPA has concluded. Members of the panel stated that the information on PON1 polymorphisms should not be used as the sole factor in a data-derived uncertainty factor for two main reasons: 1) it is only one enzyme in a complex pathway, and is subsequent to the bioactivation reaction; therefore, it can only function on the amount of bioactivation product (i.e., chlorpyrifos-oxon) that is delivered to it by CYP450): and 2) the genotype of PON1 alone is insufficient to predict vulnerability because the overall level of enzyme activity is ultimately what determines detoxication potential from that pathway; thus, it is better to use PON1 status because it provides information regarding both PON1 genotype and activity. Some of the data from laboratory animal studies in PON knockout animals are using an unrealistic animal model and frequently very high dose levels, and do not reflect what might happen in humans.

When all of the kinetic parameters including those for PON1 genotype were placed into the Timchalk and Poet PBPK model, PON1 genotype only influenced chlorpyrifos-oxon concentration at the higher doses, but did not appreciably influence chlorpyrifos-oxon levels at the lower more environmentally relevant levels. Therefore several panel members concluded that

it is not reasonable to base an uncertainty factor on PON1 genotype alone and the suggested factor of 12 is unreasonable.

One panelist stated the EPA-derived CSAF for UF_{HK} underestimated the UF HK and that a statistical approach is recommended to correct for this problem. This panelist suggested an alternative approach to calculate UF_{HK} would be to use the 99th (or 1st) percentile rather than the 95th (or 5th) to protect a greater portion of the population, and correct for the bias in estimating the GSD from the arithmetic summary statistics. The justification and approach to correcting the bias introduced when using arithmetic summary statistics is available in a manuscript currently in preparation by this panelist (Lynch et al., (2008)). An example of how this method can be applied is provided in Table 2 below. The Panel recognized that the justification for using the 99th rather than the 95th percentile is a policy decision.

Table 2: Comparison of UFHK Factors (Lynch et al. 2008)

	Default	50^{th} %tile QR Mothers/ 5^{th}%tile QQ neonates	50^{th} %tile QR Mothers/ 1st%tile QQ neonates
Current Method	3	11.6	16.5
Corrected Method	3	12.2	17.6

Additional Recommendations Regarding UFs

Following discussion of Question #7 (a-e), several panel members recommended the following:

1. Establish the UFs for chlorpyrifos based on the PoD as given in any of the three options presented in question 6, and the concern for developmental neurotoxicity as indicated in the epidemiological studies.

2. Because the Agency's choices of PoD are all based on cholinesterase inhibition (AChE inhibition), the Panel stated that it would be most appropriate that the UFs be established for this endpoint and not for the developmental neurotoxicity endpoints. Separate from establishing a set of UF for AChE inhibition endpoints, the Agency is encouraged to establish an UF specific for addressing the concerns for developmental neurotoxicity based on the evidence in the epidemiological studies.

3. Given the complexity of inter-relationship among all the TK and TD components leading to AChE inhibition, it is difficult to derive individual UFs (i.e., UF_{AD}, UF_{HD}, UF_{AT}, UF_{HT}) in a distinct and separate manner. The rate-limiting step in the formation/removal of the oxon is necessary to adequately predict the concentration of active metabolite in target tissues at environmental doses. Because of the complexity of the pathway, focusing on one enzyme that is likely not the rate-limiting step won't allow for the best, nor entire picture, of intra-

human variability estimates. Instead, the Agency is encouraged to pursue the use of a "simple" PBPK model that can integrate all key toxicokinetic and toxicodynamic factors and evaluate their contributions to the overall outcome of endpoint (e.g., AChE inhibition) or related dose metric of interest (e.g., profiles of chlorpyrifos, chlorpyrifos-oxon, and the chlorpyrifos-specific urinary metabolite, TCP) at various chlorpyrifos exposure scenarios and for various life stages. The Panel noted that the Agency has made substantial progress in exploring the use of PBPK model with a few remaining pieces yet to be completed for a preliminary review (Timchalk, 2008; comments to the docket, EPA-HQ-OPP-2008-0274). The Agency is encouraged to bring this model to a stage ready for independent peer review in the very near future, without waiting for the newly funded model. Such a model is expected to be revised as needed, and as new information becomes available in the future.

Data-derived extrapolation factors (i.e., uncertainty factors, UFs) are predicated on at least some understanding of the mode of action for the critical effect. DDEFs (also called, CSAFs) need to be based on quantitative data. At a minimum, we need to understand the rate-limiting step in delivery to the target tissue and the active metabolite. One panel member suggested this approach should be done particularly in two tissues, liver (before any amount of chlorpyrifos-oxon reaches the blood stream) and brain. There is uncertainty about whether the effect is mediated by the AChE inhibition pathway and even if so, a PBPK model is needed to characterize the relevant activation and detoxication at relevant concentrations or doses, as a basis to replace defaults.

One panel member stated that the replacement of default uncertainty factors is more often achievable for interspecies differences because of the attempt to capture ratios of central estimates for the relevant animal species and humans vs. population variability in the human population, which requires considerably more data. This panel member emphasized how misleading it is use the PON 1 activity data as a basis for replacement of default, in this case, given that it is an inappropriate surrogate for human variability.

Several panel members recommended "bounding" the reference doses developed on the basis of the animal studies, taking into account the dose-response information from the human deliberate dosing and epidemiological studies, but not in relation to the points of departure. This is because the dose-response information needs to be interpreted in the context of what we know about interspecies and intraspecies differences, such that the bounding exercise using animal studies can be tested using human data.

One panel member expressed overall concern about using PBPK models due to lack of transparency. This panelist believed that there is a blurring of inputs/outputs based on what is known (i.e., data are missing) versus what is predicted. Only when the predictions of the models can be verified with empirical data should they be used.

In the interim, the majority of the Panel recommended that the Agency apply a default UF of 100 to the PoD based on AChE inhibition; i.e., 10-fold for interspecies, 10-fold for inter-individual variation of sensitivity (i.e., intra-species differences). Some panel members recommended that the Agency should consider the use of additional UFs to address the concerns for developmental neurotoxicity as indicated in epidemiological studies. The

default UF may be modified in the future when information on the mode of action for the developmental neurotoxicity becomes available.

REFERENCES

Abou-Donia, M.B., W.A. Khan, A.M. Dechkovskaia, L.B. Goldstein, S.L. Bullman, S.L., A. Abdel-Rahman. 2006. In utero exposure to nicotine and chlorpyrifos alone, and in combination produces persistent sensorimotor deficits and Purkinje neuron loss in the cerebellum of adult offspring rats. Arch. Toxicol. 80:620–631.

Aldridge, J.E., E.D. Kevub, F.J. Seidler, T.A. Slotkin. 2005. Developmental Exposure of Rats to Chlorpyrifos Leads to Behavioral Alterations in Adulthood, Involving Serotonergic Mechanisms and Resembling Animal Models of Depression. Environment. Health Perspect. 113: 527-531.

Anderson, G.D. 2006. Using pharmacokinetics to predict the effects of pregnancy and maternal-infant transfer of drugs during lactation. Expert Opin. Drug Metab.Toxicol. 2:947-960.

Atterberry, T.T., W.T. Burnett, J.E. Chambers. 1997. Age-related differences in parathion and chlorpyrifos toxicity in male rats: Target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism. Toxicol. Appl. Pharmacol. 147:411–418.

Augustinsson, K.B. and M. Barr. 1963. Age variation in plasma arylesterase activity in children. Clin. Chim. Acta 8:568–573.

Ballough, G. P.H., R.K. Kan, J.D. Nicholson, D.M. Fath, C.P. Tompkins, G.M. Moffa, M.G. Filbert. 2008. Brain damage from soman-induced seizures is greatly exacerbated by dimethyl sulfoxide (DMSO): Modest neuroprotection by 2-aminoethyl diphenylborinate (2-APB), a transient receptor potential channel inhibitor and inositol 1,4,5-triphosphate receptor antagonist. J. Med. CBR Def. 6: 1-20. http://www.jmedcbr.org/issue_0601/Ballough/Ballough_03_08.html. Accessed December 5, 2008.

Barker, D.J.P. 2004. The Developmental Origins of Adult Disease. J. Amer. Col. Nutrition 23: 588S–595S.

Boobis, A.R., S.M. Cohen, V. Dellarco, D. McGregor, M.E. Meek, C. Vickers, D. Willcocks, and W. Farland. 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit. Rev. Toxicol. 36: 781-792.

Brophy, V.H., R.L Jampsa, J.B. Clendenning, L.A. McKinstry, G.P. Jarvik, C.E. Furlong. 2001. Effects of 5' regulatory region polymorphisms on paraoxonase-gene (PON1) expression. Am.J. Hum.Genet. 68:1428–1436.

Brzak, K.A., D.W. Harms, M.J. Bartels, R.J. Nolan. 1998. Determination of chlorpyrifos, chlorpyrifos oxon, and 3,5,6-trichloro-2-pyridinol in rat and human blood. J. Anal. Toxicol. 22: 203–210.

Buratt,i F.M, A. D'Aniello, M.T. Volpe, A. Meneguz, E. Testai. 2005. Malathion bioactivation in the human liver: the contribution of different cytochrome p450 isoforms. Drug Metab. Dispos. 33:295-302.

Buratti, F.M., C. Leoni, E.Testai. 2006. Foetal and adult human CYP3A isoforms in the bioactivation of organophosphorothionate insecticides. Toxicol. Lett. 167:245–255.

Burlina, A., E. Michielin, L. Galzigna. 1977. Characteristics and behavior of arylesterase in human serum and liver. Eur. J. Clin. Invest. 7(1): 17-20.

Butte, N.F. 2000. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am. J. Clin. Nutr. 71(5S):1256S-61S.

Carr, R.L. and C.A. Nail. 2008. Effect of different administration paradigms on cholinesterase inhibition following repeated chlorpyrifos exposure in late preweanling rats. Toxicological Sciences. In press. PMID: 18703558

Carr, R.L., H.W. Chambers, J.A. Guarisco, J.R. Richardson, J. Tang, J.E. Chambers. 2001. Effects of repeated oral postnatal exposure to chlorpyrifos on open-field behavior in juvenile rats. Toxicol. Sci. 59: 260-267.

Chambers, J.E., and H.W. Chambers. 1989. Oxidative desulfuration of chlorpyrifos, chlorpyrifos-methyl, and leptophos by rat brain and liver. J. Biochem. Toxicol. 4:201–203.

Chambers, J.E., H.W. Chambers, J.E. Snawder. 1991. Target site bioactivation of the neurotoxic organophosphorus insecticide parathion in partially hepatectomized rats. Life Sci. 48:1023-1029.

Chambers, J.E., J.R. Munson, H.W. Chambers. 1989. Activation of the phosphorothionate insecticide parathion by rat brain in situ. Biochem. Biophys. Res. Comm. 165:327-333.

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

Choi, K., H. Joo, R.L. Rose, E. Hodgson. 2006. Metabolism of chlorpyrifos and chlorpyrifos oxon by human hepatocytes. J. Biochem. Mol. Toxicol. 20:279–291.

Clancy, B., Finlay B.L., R.B. Darlington, K.J.S. Anand. 2007. Extrapolating brain development from experimental species to human. Neurotoxicology 28: 931-937.

Cole, T.B., B.J. Walter, D.M. Shih, A.D. Tward, A.J. Lusis, C. Timchalk, R.J. Richter, L.G. Costa, and C.E. Furlong. 2005. Toxicity of chlorpyrifos and chlorpyrifos oxon in a transgenic mouse model of the human paraoxonase (PON1) Q192R polymorphism. Pharmacogenet Genomics 15: 589-598.

Czekaj, P., A. Wiaderkiewicz , E. Florek, R. Wiaderkiewicz. 2000. Expression of cytochrome CYP2B1/2 in nonpregnant, pregnant and fetal rats exposed to tobacco smoke. Acta Biochim. Pol. 47:1115-1127.

Dam, K., F.J. Seidler, T.A. Slotkin. 2000. Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. Dev. Brain Res. 121: 79-189.

DeMatteis, F. 1974. Covalent binding of sulphur to microsomes and loss of cytochrome P450 during oxidative desulphuration of several chemicals. Mol. Pharmacol. 10: 849-854.

Draganov, D.I. and B.N. La Du. 2004. Pharmacogenetics of paraoxonases: a brief review. Arch. Pharmacol. 369: 78–88.

Duysen, E.G., B. Li, S. Darvesh, O. Lockridge. 2007. Sensitivity of butyrylcholinesterase knockout mice to (–)-huperzine A and donepezil suggests humans with butyrylcholinesterase deficiency may not tolerate these Alzheimer's disease drugs and indicates butyrylcholinesterase function in neurotransmission. Toxicology 233:60–69.

Eaton, D.L,, R.B. Daroff , H. Autrup, J. Bridges, P. Buffler, L.G. Costa, J. Coyle, G. McKhann, W. C. Mobley, L. Nadel, D. Neubert, R. Schulte-Hermann, P.S. Spencer. 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit. Rev. Toxicol. 38 (S2):1-125.

Ecobichon, D.J., and D.S. Stephens. 1973. Perinatal development of human blood esterases. Clin. Pharmacol. Ther. 14:41–47.

Engel, S.M., G.S. Berkowitz, D.B. Barr, S.L. Teitelbaum, J. Siskind, S.J. Meisel, J.G. Wetmur, M.S. Wolff. 2007. Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. Am. J. Epidemiol. 165 (12):1397-404.

Eskenazi, B., K. Harley, A. Bradman, E. Weltzien, N.P. Jewell, D. B. Barr, C. E. Furlong, N.T. Holland. 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Env. Health Perspect. 112 (10):1116-1124.

Eskenazi, B., A.R. Marks, A. Bradman, K. Harley, D.B. Barr, C. Johnson, N. Morga, N.P. Jewell. 2007. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect. 115 (5): 792-798.

Eskenazi, B., L.G. Rosas, A.R. Marks, A. Bradman, K. Harley, N. Holland, C. Johnson, L. Fenster, D.B. Barr. 2008. Pesticide toxicity and the developing brain. Basic Clin Pharmacol Toxicol. 102: 228-236.

Etzel, RA, ed. 2003. Pediatric Environmental Health. 2nd EditionAmerican Academy of Pediatrics. Elk Grove Village IL.

Figueras, F., J. Figueras, E. Meler, E. Eixarch, O. Coll, E. Gratacos, J. Gardosi, X. Carbonell. 2007. Customised birthweight standards accurately predict perinatal morbidity. *Arch. Dis.* Child. Fetal Neonatal Ed. 92(4): F277-F280.

Furlong, C.E. 2007. Genetic variability in the cytochrome P450-paraoxonase (PON1) pathways for the detoxication of organophosphorus compounds. J. Biochem. Mol. Toxicol. 21:197–205.

Furlong, C.E., T.B. Cole, G.P. Jarvik, C. Pettan-Brewer, G.K. Geiss, R. J. Richter, D. M. Shih, A. D. Tward, A. J. Lusis, L.G. Costa. 2005. Role of Paraoxonase (PON1) Status in Pesticide Sensitivity: Genetic and Temporal Determinants. NeuroToxicology 26(4): 651-659.

Ginsberg, G., D. Hattis, B. Sonawane. 2004. Incorporating pharmacokinetic differences between children and adults in assessing children's risk to environmental toxicants. Toxicol. Appl. Pharmacol. 198: 164–183.

Griffin, P., H. Mason, K. Heywood, J. Cocker, J. 1999. Oral and dermal absorption of chlorpyrifos: A human volunteer study. J. Occup. Environ. Med. 56:10–13.

Halpert, J., D. Hammond, R.A. Neal. 1980. Inactivation of purified rat liver cytochrome P450 during the metabolism of parathion (diethyl p-nitrophenyl phophorothionate). J. Biol. Chem. 255: 1080-1089.

Holland, N., C. Furlong, M. Bastaki, R. Richter, A. Bradman, K. Huen, K. Beckman, and B. Eskenazi, B. 2006. Paraoxonase polymorphisms, haplotypes, and enzyme activity in Latino mothers and newborns. Environ. Health Perspect. 114:985–991.

Hunter, D.L., T.L. Lassiter, S. Padilla. 1999. Gestational exposure to chlorpyrifos: comparative distribution of trichloropyridinol in the fetus and dam. Toxicol. Appl. Pharmacol. 158: 16-23.

Icenogle, L.M., C. Christopher, W.P. Blackwelder, D.P. Caldwell, D. Qiao, F.J. Seidler, T.A. Slotkin, E.D. Levin. 2004. Behavioral alternations in adolescent and adult rats caused by a brief subtoxic exposure to chlorpyrifos during neurulations. Neurotoxicol. Teratol. 26: 95-101.

Jett, D.A., R.V. Navoa, R.A. Beckles, G.L. McLemore. 2001. Cognitive function and cholinergic neurochemistry in weanling rats exposed to chlorpyrifos. Toxicol. Appl. Pharmacol. 174:89–98.

Karanth, S. and C. Pope, C. 2003. Age-related effects of chlorpyrifos and parathion on acetylcholine synthesis in rat striatum. Neurotoxicol. Teratol. 25:599–606.

Karanth, S. and C. Pope. 2000. Carboxylesterase and A-esterase activities during maturation and aging: Relationship to the toxicity of chlorpyrifos and parathion in rats. Toxicol. Sci. 58:282–289.

Kisicki, J.C., C.W. Seip, M.L. Combs. 1999. A rising dose toxicology study to determine the no-observable effect levels (NOEL) for erythrocyte acetylcholinesterase (AChE) inhibition and cholinergic signs and symptoms of chlorpyrifos at three dose levels. DowAgroSciences LLC.

Lassiter, T.L., S. Padilla, S.R. Mortensen, S.M. Chanda, V.C. Moser, S. Barone, S., Jr. 1998. Gestational exposure to chlorpyrifos: Apparent protection of the fetus? Toxicol. Appl. Pharmacol. 152:56–65.

Laviola, G. W. Adriani, C. Gaudino, R. Marino, F. Keller. 2006. Paradoxical effects of prenatal acetylcholinesterase blockade on neuron-behavioral development and drug-induced stereotypes in reeler mutant mice. Psychopharmacol. 187: 331-344.

Levin, E.D., N. Addy, A. Baruah, A. Elias, N.C. Christopher, F. J. Seidler, T.A. Slotkin. 2002. Prenatal chlorpyrifos exposure in rats causes persistent behavioral alternations. Neurotoxicol. Teratolog. 24: 733-741.

Levin, E.D., N. Addy, A. Nakajima, N.C. Christopher, F.J. Seidler, T.A. Slotkin. 2001. Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. Dev. Brain Res. 130: 83-89.

Li,W.F., C. Matthews, C.M. Disteche, L.G. Costa, C.E. Furlong. 1997. Paraoxonase (PON1) gene in mice: Sequencing, chromosomal localization and developmental expression. Pharmacogenetics 7:137–144.

Li, B., M. Sedlacek, I. Manoharan, R. Boopathy, E.G. Duysen, P. Masson, P., O. Lockridge. 2005. Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. Biochem. Pharmacol. 70:1673–1684.

Liu, J., K. Olivier, C.N. Pope. 1999. Comparative neurochemical effects of repeated methyl parathion or chlorpyrifos exposures in neonatal and adult rats. Toxicol. Appl. Pharmacol. 158:186–196.

Lynch M.K., W. Heiger-Bernays, A. Ozonoff, 2008. Quantification and Correction of the Bias in the Estimated Geometric Standard Deviation Implications for the Derivation of the Reference Dose. Manuscript in Preparation

Ma, T. and J.E. Chambers. 1994. Kinetic parameters of desulfuration and dearylation of parathion and chlorpyrifos by rat liver microsomes. Food Chem. Toxicol. 32:763-767.

Ma, T. and J.E. Chambers. 1995. A kinetic analysis of hepatic microsomal activation of parathion and chlorpyrifos in control and phenobarbital-treated rats. J. Biochem. Toxicol. 10:63-68.

March of Dimes; http://www.marchofdimes.com/professionals/14332_1153.asp. Accessed September 21, 2008.

Marty, M.S., J.Y. Domoradzki, S.C. Hansen, C. Timchalk, M.J. Bartels, J.L. Mattsson. 2007. The effect of route, vehicle, and divided doses on the pharmacokinetics of chlorpyrifos and its metabolite trichloropyridinol in neonatal Sprague-Dawley rats. Toxicol. Sci. 100:360–373.

Mattsson, J.L., J.P. Maurissen, R.J. Nolan, K.A. Brzak, K.A. 2000. Lack of differential sensitivity to cholinesterase inhibition in fetuses and neonates compared to dams treated perinatally with chlorpyrifos. Toxicol. Sci. 53:438–446.

Maurissen, J.P., A.M. Hoberman, R.H. Garman, R.H., T.R. Hanley Jr. 2000. Lack of selective developmental neurotoxicity in rat pups from dams treated by gavage with chlorpyrifos. Toxicol. Sci. 57:250–263.

Meek, M.E., J.R. Bucher, S.M. Cohen, V. Dellarco, R.N. Hill, L.D. Lehman-McKeeman, D.G. Longfellow, T. Pastoor, J. Seed, D.E. Patton. 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33: 591-653.

Meuling, W.J.A., L.C. Ravensberg, L.Roza, J.J. van Hemmen. 2005. Dermal absorption of chlorpyrifos in human volunteers. Int. Arch. Occup. Environ. Health 78:44–50.

Morgan, E.W., B. Yan, D. Greenway, A. Parkinson. 1994. Regulation of two rat liver microsomal carboxylesterase isozymes: species differences, tissue distribution, and the effects of age, sex, and xenobiotic treatment of rats. Archives Biochem. Biophysics 315(2): 513-526.

Mortensen, S.R., S.M. Chanda, M.J. Hooper, S. Padilla. 1996. Maturational differences in chlorpyrifos-oxonase activity may contribute to age-related sensitivity to chlorpyrifos. J. Biochem. Toxicol. 11:279–287.

Moser, V.C. 2000. Dose-response and time-course of neurobehavioral changes following oral chlorpyrifos in rats of different ages. Neurotoxicol. Teratol. 22:713–723.

Moser, V.C., S.M. Chanda, S.R. Mortensen, S. Padilla. 1998. Age- and gender-related differences in sensitivity to chlorpyrifos in the rat reflect developmental profiles of esterase activities. Toxicol. Sci. 46:211–222.

Nolan, R.J., D.L. Rick, N.L. Freshour, H. Saunders. 1984. Chlorpyrifos: Pharmacokinetics in human volunteers. Toxicol. Appl. Pharmacol. 73:8–15.

Nomura, D. K., K.A. Durkin, K.P. Chiang, G.B. Quistad, B.F. Cravatt and J.E. Casida. 2006. Serine hydrolase KIAA1363: Toxicological and structural features with emphasis on organophosphate interactions. Chem.Res. Toxicol. 19 , pp. 1142-1150.

Phuntuwate, W., C. Suthisisang, B. Koanantakul, M. Mackness, B. Mackness. 2005. Paraoxonase 1 status in thre Thai population. J Human Genetics 50(6): 293-300.

Poet, T.S., H. Wu, A.A. Kousba and C. Timchalk. 2003. *In vitro* hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. Toxicol. Sci. 72: 193–200.

Rauh, V.A, R. Garfinkel, F.P. Perera, H.F. Andrews, L. Hepner, D.B. Barr, R.Whitehead, D. Tang, R.M. Whyatt. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118: e1845-e1859.

Rauh, V.A. 2008. Discussion of analyses of prenatal chlorpyrifos exposure and neurodevelopmental outcomes. Presentation to US EPA (April 23, 2008).

Ricceri, L., A. Venerosi, F. Capone, M.F. Cometa, P. Lorenzini, S. Fortuna, G. Calamandrei. 2006. Developmental neurotoxicity of organophosphorous pesticides: Fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice. Toxicol. Sci. 93:105–113.

Ricceri, L., N. Markina, A. Valanzano, S. Fortuna, M.F. Cometa, A. Meneguz, G. Calamandrei, 2003. Developmental exposure to chlorpyrifos alters reactivity to environmental and social cues in adolescent mice. Toxicol. Appl. Pharmacol. 191:189–201.

Rodier, P.M. 2004. Environmental Causes of Central Nervous System. Pediatrics 113: 1076-1083

Samarawickrema, N., A. Pathmeswaran, R. Wickremasinghe, R. Peiris-John, M. Karunaratna, N. Buckley, A. Dawson, J. de Silva. 2008. Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. Clin Toxicol (Phila). 46(6): 489-95.

Schuh, R.A., P.J. Lein, A. Rondell, A. Beckles D. A. Jett. 2002. Noncholinesterase Mechanisms of Chlorpyrifos Neurotoxicity: Altered Phosphorylation of Ca^{2+}/cAMP Response Element Binding Protein in Cultured Neurons. Toxicology and Applied Pharmacology 182(2): 176-185.

Seed, J., E. Carney, R. Corley, K. Crofton, J. DeSesso, P. Foster, R. Kavlock, G. Kimmel, J. Klaunig, E. Meek, J. Preston, W. Slikker, S. Tabacova, G. Williams. 2005. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. Crit. Rev. Toxicol. 35: 663-672.

Shih, D.M., L. Gu, Y.R. Xia, M. Navab, W.F. Li, S. Hama, L.W. Castellani , C.E. Furlong, L.G. Costa, A.M. Fogelman, A.J. Lusis. 1998. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 394: 284-287.

Sirivarasaia, J., S. Kaojarerna, K. Yoovathawornb, T. Surac. 2007. Paraoxonase (PON1) polymorphism and activity as the determinants of sensitivity to organophosphates in human subjects. Chemico-Biological Interactions 168 (3): 184-192.

Slotkin, T.A., E.D. Levin, F.J. Seidler. 2006. Comparative developmental neurotoxicity of organophosphate insecticides: Effects on brain development are separable from systemic toxicity. Environ. Health Perspect. 114:746–751.

Sogorb, M.A., S. García-Argüelles, V. Carrera, E. Quesada, E. Vilanova. 2008. Human serum albumin hydrolyzes the organophosphorus insecticides paraoxon, diazoxon, chlorpyrifos-oxon and O-hexyl O-2,5-dichlorophenyl phosphoramidate. Tox. Letters. 180(Supplement 1): S29.

Sultatos, L.G., M. Shao, S.D. Murphy. 1984. The role of hepatic biotransformation in mediating the acute toxicity of the phosphorothionate insecticide chlorpyrifos. Toxicol. Appl. Pharmacol. 73:60–68.

Timchalk, C., T.S. Poet, A.A. Kousba. 2006. Age-dependent pharmacokinetic and pharmacodynamic response in preweanling rats following oral exposure to the organophosphorus insecticide chlorpyrifos. Toxicology. 220(1):13-25.

Timchalk, C., A. Busby, J.A. Campbell, L.L. Needham, D.B. Barr. 2007. Comparative pharmacokinetics of the organophosphorus insecticide chlorpyrifos and its major metabolites diethylphosphate, diethylthiophosphate and 3,5,6-trichloro-2-pyridinol in the rat. Toxicology 237:145–157.

U.S. EPA. 2001. Organophosphate Pesticides: Preliminary OP Cumulative Risk Assessment. Washington, DC: U.S. Environmental Protection Agency, Office of Pesticide Programs. Available: http://www.epa.gov/pesticides/cumulative/pra-op [accessed 30 July 2003].

Venerosi, A. et al. 2008. Neonatal exposure to chlorpyrifos affects maternal responses and maternal aggression of female mice in adulthood. Neurotoxicol. Teratol. 11 July 2008. [Epub ahead of print]

Venerosi, A., G. Calamandrei, L. Ricceri. 2006. A social recognition test for female mice reveals behavioral effects of developmental chlorpyrifos exposure. Neurotoxicol. Teratol. 28: 466-471.

Whyatt, R.M., D.B. Barr, D.E. Camann, P.L. Kinney, J.R. Barr, H.F. Andrews, L.A. Hoepner, R. Garfinkel, Y. Hazi, A. Reyes, J. Ramirez, Y. Cosme, F.P. Perera. 2003. Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect. 111 (5): 749-756.

Whyatt, R.M., D.E. Camann, P.L. Kinney, A. Reyes, J. Ramirez, J. Dietrich, D. Diaz, D. Holmes, F.P. Perera. 2002. Residdential pesticide use during pregnancy among a chohort of urban minority women. Environ. Health Perspect. 110: 507-514.

Whyatt, R.M, R. Garfinkel, L.A. Hoepner, D. Holmes, M. Borjas, M.K. Williams, A. Reyes, V. Rauh, F.P. Perera, D.E. Camann. 2007. Within- and between-home variability in indoor-air insecticide levels during pregnancy among an inner-city cohort from New York City. Environ Health Perspect. 115(3): 383-389.

Whyatt, R.M, V. Rauh, D.B. Barr, D.E. Camann, H.F. Andrews, R. Garfinkel, L.A. Hoepner, D. Diaz, J. Dietrich, A. Reyes, D. Tang, P.L. Kinney, F.P. Perera. 2004. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ Health Perspect. 112:1125-1132.

Whyatt, R.M. Discussion of analyses of prenatal chlorpyrifos exposure and birth outcomes. Presentation to US EPA (December 13, 2007).

Whyatt, R.M. Discussion of analyses of prenatal chlorpyrifos exposure and birth outcomes. Presentation to US EPA (April 23, 2008).

Whyatt, R.M. Personal communication to D. Smegal (EPA) via email, August 12, 2008.

Whyatt, R.M. Personal communication to D. Smegal (EPA) and H.A.Young (GWU, member of the SAP) via telephone, September 18, 2008.

Young, J.G., B. Eskenazi, E.A. Gladstone, A. Bradman, L. Pedersen, C. Johnson, D.B. Barr, C.E. Furlong, N.T. Holland. 2005. Association between *in utero* organophosphate exposure and abnormal reflexes in neonates. Neurotoxicology 26 (2): 199-209.

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

APPENDIX 1: Summary of Neurobehavioral Effects of Developmental Exposure to Chlorpyrifos in Rodents: 2000-2008

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal Toxicity	Pup Toxicity	AChEI		Task	Age at Test	Results
1 Maurissen et al, 2000	Rat (S-D)	GD6 - LD 10	0.0	Gavage (corn oil)	5 mg/kg: ↓ Wgth Gain (End of Gest.)	5 mg/kg: Delay in vaginal opening	GD 20 Dams: 1 & 5 mg/kg	↓ Brain, Plasma, RBC	**Delayed Spatial Alternation: Repeated Testing**		
			0.3		Dams: High dose: muscle twitches, hypernea, hyperactivity Controls & low dose:sporatic hyperactivity	Decreased BW: Males PND 22-65	0.3 mg/kg	↓ Plasma	7 x 12	PND 23-24	Acquisition: No Rx Effect
			1.0						4 x 12	PND 24	Retention: No Rx Effect
			5.0						6 x 10	PND 72-83	Acquisition: No Rx Effect
									6 x 6	PND 85-90	Retention: No Rx Effect
								Apparatus:Wire bottom cages	**Motor Activity**	PND 13	No Rx
									(Habituation)	PND 17	No Rx
									(Beam Breaks) 1 Hr	PND 21	No Rx
									No Gender Diff	PND 60	No Rx
									Auditory Startle	PND 22	↑ lat to peak response (p=.03) [overall effect]
									50 trls: 120dB	PND 61	No Rx effects
Slotkin's Group											
2 Levin et al, 2002	Rat (S-D)	GD 17-20 Pk period neurogenesis	DMSO(1 ml/kg)	sc (DMSO)	No change in maternal care taking		N.D.		**A. Spontaneous Alternation (T-Maze)**		
			1.0						Percent Alternation	4-6 wks	No Rx Effect
			5.0						Latency to Choice 5 x 5	1 & 5 mg/kg	↑ during Trails 1 and 2 (Hyperactivity)
									B. Fig 8 Locomot. Act 3 x 1 hr	4 - 6 wks	
									Motor Activity	No Rx Effect	
									Habituation	1 & 5 mg/kg	↓ (Females)
									C. RAM (16 arms) 16 sessions	8-13wks	
									Working Memory Errors	1 mg/kg	↑ (Females)
									Reference Memory Errs.	1mg/kg	↑ (Females)
									Response Latencies	1 & 5 mg/kg	↑
									Drug Challenge(RAM)	14-17wks	
									Scopolamine	↑ in WME & RME	
									Mecamylamine	No Rx Effect	(Difference in WME bet 1 & 5 mg/kg gps)

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal Toxicity	Pup Toxicity	AChEI
3 Icenogle et al, 2004	Rat (S-D)	GD 9-12 Form/Clos Neural Tube	1.0 5.0	sc (DMSO)	None	No effects of growth/viability	N.D.

	Task	Age at Test	Results		
	Spontaneous Alternation				
A.	**(T-Maze)**	4-8 Wks			
	Percent Alternation	Not Reported			
	Latency to Choice	CPF 1 & 5	↓ (Trial 1)		
B.	**Fig 8 Locomot. Act**	4-8 Wks PND 1-4, & PND			
	SMA	11-14			
	Habituation	CPF 5	↑		
C.	**RAM (16 arms)**	8-13 wks			
	18 sessions				
	Working Memory Errors	CPF 5	↑ (Early Trials)		
	Reference Memory Errs.	CPF 5	↑ (Early Trials)		
	Drug Challenge(RAM)	14-17wks			
	Scopolamine	CPF 5	Attenuated response		
	Mecamylamine		No Effects		
D	**Startle/ PPI**		No Rx Effects		
	65 Db Bckg: 110dB SS PP 68,71,77dB				
E	**Elevated Plus Maze**				
	Center crosses	CPF 5	↑		

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal Toxicity	AChEI
4 Levin et al, 2001	Rat (S-D)	PND 1-4	1.0	sc (DMSO)	None	N.D.
		PND 11-14	5.0	sc (DMSO)		

	Task	Age at Test	Results		
	Spontaneous Alternation				
A.	**(T-Maze)**	4-6 Wks PND 1-4, PND 11-			
	Percent Alternation	14	No Rx Effect		
	Latency to Choice	PND 1-4, & PND 11-14	↓ (Males)		
B.	**Fig 8 Locomot. Act**	4-6 Wks PND 1-4, & PND			
	SMA	11-14	No Rx Effect		
	Habituation	PND 11-14	↓ (For first session)		
C.	**RAM (16 arms)**	8-13 wks			
	18 sessions				
	Working Memory Errors	PND 1-4(CPF1)	↓ (Fems)	↑ Males	
	Reference Memory Errs.	PND 1-4	↓ (Fems)	↑ Males	Initial phase
		PND 11-14	No Rx Effect		
	Drug Challenge(RAM)	14-17wks			
	Scopolamine	No scop impair (CPF1 Fems) WME			
	Mecamylamine	No RX effect			

	Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal/Pup Toxicity	AChEI		Task	Age at Test	Results	
5	Aldridge et al, 2005	Rat (S-D)	PND 1-4	1.0	sc (DMSO)	None			A. Elevated Plus Maze	PND 52-53		
									Time in Open Arms		↑ (Males)	
									Center crosses		↑ (Males)	
									B Choc Milk Consumpt	PND 54		
									2 choice/ 2 hrs	Preference	↓(M & F)	
									C RAM	PND 64-97		
									Working Memory Errors		↓ (Fems)	↑ Males
									Reference Memory Errs.		↓ (Fems)	↑ Males
									Drug Challenge(RAM)	16-17wks		
									Ketaserin	WME	↑ (M & F)	
									5-HT2 antagonist	RME	↑ (M & F)	
							Acute Study					
							2 hr aft first Rx PND 1		Reflexes			
6	Dam et al, 2000	Rat (S-D)	PND 1-4	1.0	sc (DMSO)	None						
							1 mg/kg Males	↓60% (CBM, BS, FBr). Rec. 4 hr	Righting Reflex	PND 3-4		
			PND 11-14	5.0	sc (DMSO)		1 mg/kg Females	↓20% (CBM, BS, FBr)	Time to Right	CPF 1	↑ Females	
							2 hr aft CPF Rx PND11		Negative Geotaxis	PND 5-8		
							5 mg/kg	↓20% (CBM, BS, FBr) M & F.	Pups meeting ctiteria	CPF 1	↓ Females	
									Open Field	PND 21		
										SMA	↓ Males	
										Rearing	↓ Males	
										PND 30		
										SMA	↓ Males	
										Rearing	↓ Males	

Calamandrei's Group

	Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal/Pup Toxicity	AChEI		Task	Age at Test	Results
7	Venerosi et al, 2006	CD-1 Mice	GD 15-18 and PND 11-14	0,3.0, or 6.0 & 0, 1.0, 3.0	Gavage (peanut oil)	None	GD 19: CPY 3 & 6	20-40% I serum AchE (at 24 but not 48 hrs after Rx)	Social Recognition Test	4 months GD CPF6	↑ Rate of USV
									Retest Same Partner	GD CPF6	↑ Rate of USV
									Retest Different Partner.	GD CPF6	↑ Rate of USV
									Social Investigation Test	GD CPF6	↑ Social investigation

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal/Pup Toxicity	AChEI	Task	Age at Test	Results
8 Venerosi et al, 2008	CD-1 Mice	PND 11-14	3.0	sc (peanut oil)	Maternal Behavior: PND 1: Decreased latencies to first lick PND 2:Light-Dark Test: Decreased lat. to enter light comp PND 7: Dec aggression; Inc investigation of intruder PND 1-7: Nest Building: Increases Latencies PND7: Maternal Agression		Social Approach Social Novelty	PND 45	↓ Social contact (Session 1) No Rx Effect
9 Ricceri et al, 2006	CD-1 Mice	GD 15-18 and PND 11-14	0,3.0, or 6.0 & 0, 1.0, 3.0	Gavage (peanut oil) sc (peanut oil)	None	Pups GD 19: Dose dependent decrease(Serum) No Rx effect: Brain PND 15: Same as GD 19	Open Field (SMA) Isolation- induced aggression Maternal Behavior Elevated Plus Maze	PND 70(Males) PND 75-80 (Males) PND 90 (Fems) PND 120 (M&F) Time in Open arms Head Dipping	↑ CPF 6 ↑ Dur Attack (GD CPF3) ↑ Off posture (CPF6) Def post: No Rx effect ↑licking dura & crouching (PN CPF 1&3) ↓Licking freq (PN CPF 1&3) ↑ PN CPF3 Fems ↓ PNCPF 3 Males
10 Ricceri et al, 2003	CD-1 Mice	PND 1-4, or PND 11-14 PND 32-35	1.0, 3.0 1.0, 3.0 1.0, 3.0	sc (DMSO)	No overt signs of chlinergic intoxication at either age	PND 4: CPF 1 & CPF 3 — Non dose-depend. AChel (total & soluble AChE) 1hr but not at 4 & 24 hrs	**Neonates** USV (Social) Homing (Olfactory) Locomotor Activity **Adolescents** Novelty Seeking Social Interaction Agonist Behavior **Adult** Passive Aviodance	 PND 5,8,11 PND 10 PND 25 PND 35 PND 45 PND 45 Males PND 60 Males	 No Rx Effect: CPF 1-4 or CPF 11-14 No Rx Effect: CPF 1-4 or CPF 11-14 No Rx Effect: CPF 1-4 or CPF 11-14 CPF 1-4 ↑ Activity No Rx effect: novelty seeking CPF 11-14 ↑ Activity No Rx effect: novelty seeking CPF 1-4 ↓ Self Grooming CPF 11-14 ↑ Soliciting resp. CPF 1-4 ↑ Agonistic resp. CPF 11-14 ↑ Agonistic resp. CPF 1-4 No Rx effect CPF 11-14 No Rx effect

Individual Studies

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal Toxicity	Pup Toxicity	AChEI	Task	Age at Test	Results
11 Moser, 2000	Rat (Long-Evans)	PND 17 PND 27 PND 70	4, 10, 20 mg/kg 10, 25, 50 mg/kg 10, 50, 100 mg/kg	Oral gavage (corn oil)	N/A	Weight losss in all groups	N.D.	**FOB (Functional Observational Battery)** Neuromuscular Activity/reactivity Sensorimotor Physiological	 PND 70 PND 17 PND 27 PND 70 PND 17 PND 27 PND 70 PND 17 PND 27 PND 70	Forelimb Grip Foot Splay ↑ (F) (CPF 50) ↑ (F)(CPF 100) Total Activity Rearing Arousal Handl. React ↓ (M & F) (CPF 10) ↓(F) (CPF 20) ↓ (M & F) (CPF 25) ↓ (M & F) (CPF 25) ↓(M & F) (CPF 50/25) ↓(F) (CPF 50) ↓(M & F) (CPF 10/50) ↓ (M & F) (CPF 50) ↓(M & F) (CPF 50/100) ↓(M) (CPF 100) Tail-pinch Response Click Response Touch Response ↓ (M & F) (CPF 10/4) ↓ (M) (CPF 20) ↓ (M & F) (CPF 50/25) ↓ (M) (CPF 25) ↓(F) (CPF 25) ↓ (M & F) (CPF 10/4) ↓ (M & F) (CPF 50) ↓ (M) (CPF 50) Weight Temperature ↓(F) (CPF 25) ↓ (M & F) (CPF 25) ↓ (M & F) (CPF 50) ↓ (M & F) (CPF 50)

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal Toxicity	Pup Toxicity	AChEI		Task	Age at Test	Results
12 Carr et al, 2001	Rat (S-D)	PND 1-21	LOW: 3/mg/kg (Every 2nd Day)	Gavage (corn oil)	N/A	↓ Body Weight, PND 13-21 (HIGH Dose)	PND 6, 10, 16 20,25, 30: ~ 50% Inhibition in forebrain and hindbrain in all 3 Rx groups	Heart: ChEI (Low Dose: PND 6,10,16,20). (Med Dose: PND 10, 16, 20, 25). (High Dose: PND 16, 20, 25). Diaphragm: (Low Dose: PND 6,10,16). (Med Dose: PND 10, 16, 20). (High Dose: PND 16, 20, 25). Serum AChEI: (Low Dose: PND 6,10). (Med Dose: PND 10, 16, 20). (High Dose: PND 16, 20).	Open Field Activity	PND 10-20	No Rx Effect
		PND 1-5, 7-21	MED: 3 mg/kg/day, 6 mg/kg/day				Skeletal Muscle: ChEI on PND 6 (Low Dose); PND 10, 16(Low & Med Doses)			PND 25 & 30	↓ High & Med Doses M & F(Dose-related)
		PND 1-5, 7-13, 15-21	HIGH: 3 mg/kg/day, 6 mg/kg/day, 12 mg/kg/day				Lung: ChEI on PND 6 (Low Dose); PND 10, 16, 20 (Low & Med Doses)				

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal Toxicity	Pup Toxicity	AChEI		Task	Age at Test	Results
13 Jett et al, 2001	Rat (Long-Evans)	PND 7, 11, 15	0.3 or 7.0	s.c. (peanut oil)	N/A	No overt signs of cholinergic toxicity	Cortex, hippocampus, cerebellum: PND 7,8 16, 28 (Pre/Post)	No ChEI due to CPF Rx	Morris water maze	PND 24-28	
		PND 22, 26	0.3 or 7.0							Acquisition	Preweaning CPF 7.0: ↑escape latency Day 5
							Mucarinic Receptor Binding	No Rx Effects			↓ percentage of rats finding platform by Day 5
										Probe	Preweaning CPF 7.0:↓ time in platform training quadrant
										PND 24-28	
										Acquisition	Postweaning CPF 0.3 & 7.0: ↑escape latency Day 5 ↓percentage of rats finding platform by Day 5
										Probe	Postweaning CPF 0.3 & 7.0: ↓ time in platform training quadrant
14 Abou-Donia et al, 2006	Rat (S-D)	GD 4-20	1.0	Dermal in 70% Ethanol	No Overt signs of tocicity in dams or pups		PND 90:			PND 90	
					No difference in litter size	No differences in body weight			Beam Walking (Coordination)	Walk time: No Rx Effect	
							Midbrain	No Rx Effect			
							Cerebellum	↑ in AChEI (F)	Inclined Plane (Sensorimotor Reflexes)	↓ in fall angle (F)	
							Brainstem	↑ in AChEI (F)			
									Forepaw Grip Time (Motor Strength)	↓ in grip time (M & F)	

Article	Species	Exposure Period	Dose (mg/kg/day)	RoA	Maternal Toxicity	Pup Toxicity	AChEI	Task	Age at Test	Results
15 Laviola et al, 2006	Wild-type Reeler mice	GD 14, 15, 16	5 mg/kg CPF-oxon	Osmotic mini pump (DMSO): Inserted under neck skin of dam	None (WT only)	None (WT only)	PND 10-12 WT type valuse used as the 100% level	Ultrasonic Vocalizations (Social Assessment)	PND 3, 7, 11	No Rx effect
N.B.: Only data from wild type mice are presented.								Grasping Reflex (Sensory/Motor)	PND 3, 7, 11	↑ in fall angle
								Righting Reflex (Motor)	PND 3, 7, 11	No Rx effect
								Locomotor Activity: Open Field	PND >70	No Rx effect
								Scopolamine Challenge	PND >70	↓ in Scop-induced locomotor stimulation
								Amphetamine Challgene	PND >70	↓ in Amph. induced locomotor stimulation