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

OFFICE OF
CHEMICAL SAFETY AND POLLUTION
PREVENTION

July 20, 2016

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the April 19-21, 2016 FIFRA SAP Meeting Held to Consider and Review Scientific Issues Associated with "Chlorpyrifos: Analysis of Biomonitoring Data"

TO: Jack Housenger
Director
Office of Pesticide Programs

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THRU: Laura E. Bailey, M.S.
Supervisory Physical Scientist/Executive Secretary FIFRA SAP
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Stanley Barone, Ph.D.
Acting Director
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Please find attached the meeting minutes of the April 19-21, 2016 FIFRA SAP open public meeting held in Arlington, VA. The report addresses a set of scientific issues associated with Chlorpyrifos: Analysis of Biomonitoring Data.

Enclosure

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FIFRA Scientific Advisory Panel Minutes No. 2016-01

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding Chlorpyrifos: Analysis of Biomonitoring Data

April 19-21, 2016
FIFRA Scientific Advisory Panel Meeting
Held at the
EPA Conference Center
Arlington, VA

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The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (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 scientific advice, information, and recommendations to the EPA Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The meeting minutes represent the views and recommendations of the FIFRA SAP and do not necessarily represent the views and policies of the EPA or of other agencies in the Executive Branch of the Federal government. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use. The meeting minutes do not create or confer legal rights or impose any legally binding requirements on the EPA or any party.

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NOTICE

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (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 (EPA), 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. The meeting minutes have been written as part of the activities of the FIFRA SAP. In preparing the meeting minutes, the FIFRA SAP carefully considered all information provided and presented by EPA, as well as information presented in public comment.

The meeting minutes of the April 19-21, 2016 FIFRA SAP meeting held to consider and review scientific issues associated with "Chlorpyrifos: Analysis of Biomonitoring Data" were certified by James McManaman, Ph.D., FIFRA SAP Session Chair, and Fred Jenkins, Ph.D., FIFRA SAP Designated Federal Official, on July 15, 2016. The minutes were prepared by Scott Lynn, Ph.D., FIFRA SAP Designated Federal Official and reviewed by Laura E. Bailey, M.S., FIFRA SAP Executive Secretary. The minutes are publicly available on the SAP website (https://www.epa.gov/sap) under the heading of "Meetings" and in the public e-docket, Docket No. EPA-HQ-OPP-2016-0062, accessible through the docket portal: http://www.regulations.gov. Further information about FIFRA SAP reports and activities can be obtained from its website at https://www.epa.gov/sap. Interested persons are invited to contact Fred Jenkins, Ph.D., SAP Designated Federal Official, via e-mail at jenkins.fred@epa.gov.

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SAP Minutes No. 2016-01

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

Chlorpyrifos: Analysis of Biomonitoring Data

April 19-21, 2016
FIFRA Scientific Advisory Panel Meeting
Held at
One Potomac Yard
Arlington, Virginia

James McManaman, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel

Date: JUL 15 2016

Fred Jenkins, Jr., Ph.D.
Designated Federal Official
FIFRA SAP Staff

Date: JUL 15 2016

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PANEL ROSTER

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LIST OF ACRONYMS USED

Abbreviation	Description
AChE	Acetylcholinesterase
ADME	Absorption, distribution, metabolism, excretion
AHETF	Agricultural Handler Exposure Task Force
AOP	Adverse outcome pathway
BMD	Benchmark dose
CCCEH	Columbia Center for Children's Environmental Health
CLIA	Clinical Laboratory Improvement Amendments
CPF	Chlorpyrifos
CPFO	Chlorpyrifos-oxon
DAS	Dow Agro Sciences
EPA	Environmental Protection Agency
ERDEM	Exposure Related Dose Estimating Model
FIFRA	Federal Insecticide Fungicide Rodenticide Act
FQPA	Food Quality Protection Act
GLP	Good Laboratory Practices
HHRA	Human health risk assessment
IRIS	Integrated Risk Information System
LOD	Level of detection
MOA	Mode of action
ND	Non-detect
NRC	National Research Council
OP	Organophosphate
PBPK	Physiologically based pharmacokinetic
PDP	Pesticide Data Program
PHED	Pesticide Handlers Exposure Database
PK	Pharmacokinetic
PoD	Point of departure
RBC	Red blood cell
RfD	Reference dose
SAP	Science Advisory Panel
TCPy	3,5,6,-trichloro-2-pyridinol
TWA	Time weighted average
US	United States
WMI	Working memory index

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INTRODUCTION

On April 19-21, 2016 the US EPA Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) met in an open public meeting in Arlington, VA to consider and review scientific issues associated with "Chlorpyrifos: Analysis of Biomonitoring Data". Chlorpyrifos (*0,0*-diethyl-*0*-3,5,6-trichloro -2-pyridyl phosphorothioate) is a broad-spectrum, chlorinated organophosphate (OP) insecticide, acaricide, and miticide used to control a variety of insects. Currently, registered uses include food and feed crops, golf course turf, greenhouses, non-structural wood treatments (such as utility poles and fence posts), ant bait stations, fire ant mounds, a USDA quarantine use for containerized/potted ornamentals, and use as an adult mosquitocide. The Agency has taken a stepwise, objective and transparent approach in evaluating, interpreting, and characterizing the strengths and uncertainties associated with all of the available lines of scientific information related to the human health effects of chlorpyrifos. Like other OPs, chlorpyrifos binds to and phosphorylates the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems. This can lead to accumulation of acetylcholine and, ultimately, at sufficiently high doses, to clinical signs of toxicity. In addition to the AChE inhibiting potential of chlorpyrifos, there is a growing body of literature with laboratory animals (rats and mice) indicating that gestational and/or early postnatal exposure to chlorpyrifos may cause persistent effects into adulthood along with epidemiology studies which have evaluated prenatal chlorpyrifos exposure in mother-infant pairs and reported associations with neurodevelopment outcomes in infants and children. The Agency has followed up on previous SAP recommendations and used a physiologically based pharmacokinetic (PBPK) model to further assess the epidemiological data and the Agency is now soliciting comment on changing the critical effect for quantitative risk assessment from AChE inhibition to neurodevelopmental outcomes. Thereby the Agency would be changing the Point of Departures (PoDs) from doses eliciting 10% red blood cell (RBC) AChE inhibition to adverse effects changes in neurodevelopment as measured by epidemiology studies conducted by Columbia Center for Children's Environmental Health (CCCEH). The FIFRA SAP was convened to provide advice to the Agency regarding the evaluation of biomonitoring chlorpyrifos data from epidemiology studies.

US EPA presentations were provided during the FIFRA SAP meeting by the following (listed in order of presentation):

Welcome and Opening Remarks – Jack Housenger, Director, Office of Pesticides Programs (OPP), EPA

Chlorpyrifos: Background and Overview – Dana Vogel, Division Director, Health Effects Division (HED), OPP, EPA

Chlorpyrifos: Overview of EPA Epidemiological Literature Review – Elizabeth Holman, Dr.PH., HED, OPP

Interpreting Biomarker Data in the CCCEH Cohort Using a Physiologically Based Pharmacokinetic (PBPK) Model – Cecilia Tan, Ph.D., HED, OPP

Chlorpyrifos: Evaluation of CCCEH Blood Data and Predicted Exposures – Wade Britton, M.P.H., HED, OPP; Rochelle Bohaty, Ph.D., Environmental Fate and Effects Division (EFED), OPP; Danette Drew, HED, OPP

Point of Departure, Intra-species Extrapolation, the FQPA 10X Safety Factor, and Case Studies: Linking Exposure Assessment with PBPK Modeling to Derive Predicted Internal Doses – Anna Lowit, Ph.D., HED, OPP, EPA

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PUBLIC COMMENTS

Oral statements were provided by:

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2) Wendy Hessler, Private citizen
3) Scott Schertz, Schertz Aerial Service, Inc.
4) CropLife America
5) Ellen Webb, MPH, Center for Environmental Health
6) Scott Slaughter, The Center for Regulatory Effectiveness
7) Elliot Gordon, Ph.D., DABT, Elliot Gordon Consulting, LLC
8) Sarada Tangirala, National Campaigns Manager, Women's Voices for the Earth
9) Jennifer Sass, Ph.D., Senior Scientist, Natural Resources Defense Council
10) Exponent
11) Virginia Ruiz, Farmworker Justice
12) Sheryl H. Kunickis, Ph.D., Director, US Department of Agriculture Office of Pest Management Policy
13) Paul Hinderliter, Ph.D., Senior Scientist, Syngenta Crop Protection, LLC and Ellen T. Chang, Sc.D., Exponent, Inc.
14) Lynne Parsons Heilbrun, MPH, Faculty Specialist, Department of Family and Community Medicine, UT School of Medicine, UT Health Science Center at San Antonio

Written public comments were provided by:

1) On behalf of themselves:
   a. Dr. Michael Goodman, Emory University
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hhh. Ho Luong Tran, M.D., MPH, President and CEO, National Council of Asian Pacific Islander Physicians
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   b. Almond Hullers & Processors Association
   c. American Farm Bureau Federation
   d. AmericanHort
   e. American Soybean Association
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   j. California Citrus Quality Council
   k. California Cotton Ginners Association
   l. California Cotton Growers Association
   m. California Date Commission
   n. California Dried Plum Board
   o. California Fig Advisory Board
   p. California Fresh Fruit Association
   q. California Specialty Crops Council
   r. California Strawberry Commission
   s. California Walnut Commission
   t. Cranberry Institute
   u. CropLife America
   v. Florida Fruit & Vegetable Association

w. Golf Course Superintendents Association of America
x. National Agricultural Aviation Association
y. National Association of State Departments of Agriculture
z. National Association of Wheat Growers
aa. National Corn Growers Association
bb. National Cotton Council
cc. National Council of Farmer Cooperatives
dd. National Pest Management Association
ee. National Potato Council
ff. National Sorghum Producers
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   d. Minako Watabe, OB/GYN, Ventura County Hospital
   e. Sarada Tangirala, National Campaigns Manager, Women's Voices for the Earth
   f. Wendy Hessler, Science Communicator
15) On behalf of themselves:
   a. Natural Resources Defense Council
   b. Farmworker Justice
   c. Earthjustice
   d. Pesticide Action Network

e. United Farm Workers
f. California Rural Legal Assistance Foundation
g. Pineros y Campesinos Unidos del Noroeste

16) William Banner, M.D., Ph.D., FAAP, FAACT, FACMT, FCCM, Medical Director, Oklahoma Poison Control Center
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25) Anonymous
26) Dow AgroSciences, LLC
27) Elliot Gordon, Ph.D., DABT, Elliot Gordon Consulting, LLC
28) On Behalf of CropLife America:
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OVERALL SUMMARY

The FIFRA SAP was convened to provide advice to the Agency regarding the evaluation of biomonitoring chlorpyrifos data from epidemiology studies. The Panel supports the use of measured maternal chlorpyrifos blood concentrations as a surrogate for fetal exposure presuming chlorpyrifos readily crosses the placenta. However, there are several methodological issues/uncertainties in the analyses suggesting that a 1:1 ratio of concentrations between mother's blood and the newborn cord blood may be in error. Plasma is not the same as whole blood, but plasma was used for measurements and the physiologically based pharmacokinetic (PBPK) models are built using whole blood. Most of the Panel was not in favor of using cord blood chlorpyrifos levels as a surrogate for exposure of infants (<1 year old). The use of the cord blood measurements to inform an ex-utero infant's exposure-response to chlorpyrifos raised many concerns, resulting in greatly diminished support. Most of the Panel was not in favor of using the chlorpyrifos concentration in cord blood at birth as a surrogate for the chlorpyrifos blood concentration during the 1- to 2-year age period.

The Panel agrees with the Agency that there are complex unknown variables that are recognized as uncertainties. Because many uncertainties cannot be clarified, the majority of the Panel does not have confidence that the Columbia Center for Children's Environmental Health (CCCEH) cord blood data on chlorpyrifos concentrations can accurately be used in quantitative risk assessment to determine a Point of Departure (PoD). A major source of uncertainty for the Panel was the lack of verification and replication of the analytical chemistry results that reported very low levels of chlorpyrifos (pg/g). Imputing quantitative values when the concentration of analyte falls below the level of detection (LOD) was a particular concern, especially given that a large fraction of cord blood samples included in the analyses presented with levels below LOD.

Multiple Panel members noted that PBPK modeling is a valuable tool to interpret the biomonitoring data in circumstances where multiple routes of exposure occur and when based on best available information as inputs. Concern was raised by at least two Panel members about parameter use, model assumptions, and an absence of sensitivity analysis. Panel members were not in consensus as to the level of agreement between the Agency's exposure characterization of the CCCEH and the blood measurements from the study. Overall, the Panel found that the general scenarios provided for PBPK modeling are reasonable (drinking water, food, residential). The Panel found several sources of uncertainty in the estimates of internal blood levels and their relationship to the CCCEH cohort results. Some Panel members thought the quality of the CCCEH data is hard to assess when raw analytical data have not been made available, and the study has not been reproduced.

The Panel agrees that both epidemiology and toxicology studies suggest there is evidence for adverse health outcomes associated with chlorpyrifos exposures below levels that result in 10% red blood cell (RBC) acetylcholinesterase (AChE) inhibition (i.e., toxicity at lower doses). However, the Panel agrees with the Agency that applying additional safety factors to the AChE PoDs to account for a possible noncholinergic mode of action (MOA) would be problematic because of challenges in justifying any particular value for such an adjustment. The majority of the Panel did not agree with the Agency's use of the results from a single longitudinal study to make a decision based on

the use of cord blood measures of chlorpyrifos as a PoD for risk assessment. The Panel was not in favor of using the lower limit of the top tertile for deriving a PoD for extrapolating risk to chlorpyrifos. The Panel was not satisfied with the arbitrary cut-off for the tertile distributions, the high-low dichotomization in the analysis, and the differences in interpretation based on the high-low dichotomization compared to that obtained when grouped into a non-detect (ND), low, mid, and high groups. The Panel considers the Agency's decision to use Benchmark Dose (BMD) derived from linear regression for deficits in working memory as a valid plan if the Agency were to decide to use cord blood chlorpyrifos to determine PoD. The majority of the Panel stated that using cord blood chlorpyrifos concentrations for derivation of the PoD could not be justified by any sound scientific evaluation. The Panel was conflicted with respect to the importance of a 2% change in working memory.

Overall the Panel found that the use of the 10X intra-species extrapolation factor is appropriate, however there are other areas of uncertainty that should be considered. Uncertainty is mostly attributed to the inability of single measures of chlorpyrifos concentration in blood to provide information regarding source, frequency, duration and magnitude of exposure, and how these exposures are linked to specific outcomes measured in the CCCEH study participants. Overall, the Panel concurs with the Agency's conclusion that the lack of postnatal exposure assessment in the CCCEH study is a source of uncertainty. While the prenatal period is likely the most sensitive to chlorpyrifos exposure, the brain continues rapid development postnatally. The majority of Panel members stated that the moderate sample size of the CCCEH is not a source of uncertainty, however some Panel members did identify uncertainties. Some Panel members stated that the sample size may have limited the CCCEH study's ability to examine the association of chlorpyrifos blood concentration on neurodevelopment in more vulnerable populations, such as racial minorities or lower socioeconomic status individuals.

The Panel decided the model was appropriately implemented with regard to dose linearity and the duration of the simulations. The simulated blood concentrations were in the pg/mL range, so saturation of metabolism was not a concern. Other model parameter values such as tissue / blood partition coefficients and blood flows to tissues were unlikely to be affected by the relatively low chlorpyrifos blood concentration, so the assumption that internal concentration would be linearly related to external dose rate was appropriate. Simulations of the various exposure scenarios were run well into the steady-state time frame. The inputs of chlorpyrifos to the PBPK model from drinking water, food, and worker – handler exposure appeared to be appropriate and defensible. The inputs were well informed by a considerable amount of data relating to expected drinking water concentrations, and dietary contributions. Worker – handler inputs were informed by highly developed occupational exposure methodologies and exposure data sources that have been extensively peer reviewed. There were no compelling issues with regard to the suitability of the inputs. If the simulated internal chlorpyrifos concentrations and the proposed Reference Dose (RfD) were realistic, large adverse effects should be seen in babies born to women who are worker – handlers of chlorpyrifos or who experience the food-borne chlorpyrifos input scenario. The food exposure scenario suggested that almost 90 percent of the adult female population always would have blood chlorpyrifos concentrations above the proposed RfD.

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EXECUTIVE SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

Question 1 – Lifestages for Consideration (Section 4)
Fetuses may be exposed to chlorpyrifos through the mother, while infants and children are exposed directly through dietary exposure (food and drinking water). The Agency has conducted case studies to use cord blood data as a surrogate to evaluate the health impacts on fetuses and infants from exposures to chlorpyrifos (Section 6). Based on the available data, there are several assumptions that are being made in order to assess exposure for specific lifestages.

> **Question 1a. Fetal exposure** – Without a gestational model that is parameterized with chlorpyrifos information, fetal exposure cannot be assessed directly. However, maternal and cord blood concentrations of chlorpyrifos from CCCEH are highly correlated (Figure 1), and preliminary evaluation of the Dow gestational PBPK model suggests little differences in blood levels between pregnant and non-pregnant women. Therefore, the Agency has concluded that the parameterized model which is available for females (13-49 years old) can be used as a reasonable surrogate for estimating fetal exposure.
> ***Please comment on the Agency's proposal to use female blood levels as a surrogate for fetal exposure.***

Panel Summary
The Panel supports the use of female blood levels as a surrogate for fetal exposure. Specifically, the measured fetal cord blood concentrations track the mother's measured blood concentrations. This suggests that chlorpyrifos readily crosses the placenta, thus the fetal cord blood and the rest of fetal systemic circulation are assumed to track the mother's blood. However, there are several methodological issues/uncertainties in the analyses of the mother:newborn cord blood ratio, such as the timing of sample collection and how non-detects were handled, suggesting that a 1:1 ratio of concentrations between mother's blood and the newborn cord blood may be in error. Much uncertainty would be removed if the raw data and sampling times were provided for reanalysis. Plasma was used for measurements, not whole blood. This needs to be corrected because the PBPK models are built using whole blood, not plasma.

> **Question 1.b. Infant (<1 year old) exposure** – Studies of chlorpyrifos in laboratory animals do not suggest any specific critical period or lifestage, but instead suggest both pre- and post-natal periods of susceptibility. In contrast, there are limited epidemiological evidence regarding postnatal exposure to chlorpyrifos or other OPs to infants and children. Because brain development continues through childhood, and due to the concern that AChE inhibition may not be protective of the neurodevelopment outcomes, the Agency is proposing to use the chlorpyrifos cord blood data from CCCEH as the most relevant source of information for deriving a PoD for infants (see Appendix 4.0 for further details).
> ***Please comment on the Agency's proposal to use cord blood data as a surrogate for assessing infant exposure.***

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Panel Summary
Most of the Panel was not in favor of using cord blood chlorpyrifos levels as a surrogate for exposure of infants (<1-year-old). One Panel member indicated that the fetal cord blood samples can be used as a PoD for infants while another Panel member suggested cord blood measurements may, with caveats, serve as a surrogate for the neonate only. The use of the cord blood measurements to inform an ex-utero infant's exposure-response to chlorpyrifos raised many concerns, resulting in greatly diminished support. The pharmacokinetics and pharmacodynamics of chlorpyrifos in an infant are largely unknown; thus, using cord plasma concentrations of chlorpyrifos to target infant plasma concentrations is not advisable. The robustness of the PBPK model to predict plasma concentrations of chlorpyrifos is moderate, at best, because of a lack of biomonitoring data and exposure information. One panel member, however, contended that the CCCEH study information on chlorpyrifos cord blood would be a useful and relevant source of information for deriving a PoD as it would be a conservative estimate of, what is likely to be higher, exposure in infants.

> **Question 1.c. Children (ages 1<2 years old) exposure** – At this time, the Agency has not included a case study for evaluating the health impacts on children 1<2 years old. However, the Agency is aware that this age group often has the highest exposure from food consumption (Section 4), as is the case for some food commodities for chlorpyrifos exposure. Children 1<2 years old have not yet been included in the case studies as these ages are temporally removed from gestational exposure; as such the relevance of the cord blood data to predict the outcomes in toddlers is unclear.
> ***Please comment on the strength and uncertainties of using the CCCEH cord blood data as a surrogate for assessing (children ages 1<2 years old) exposure to chlorpyrifos.***

Panel Summary
Most of the Panel was not in favor of using cord blood as a surrogate for the infant between 1- and 2-years of age. Many of the answers given to Charge Question 1.b. also apply to Charge Question 1.c. Given the ~5-day terminal half-life of chlorpyrifos, it would seem unreasonable to think that the chlorpyrifos concentration in cord blood at birth would directly influence the chlorpyrifos blood concentration between 1 and 2 years of age. However, one panel member contended that the CCCEH study information on chlorpyrifos cord blood would be a useful and relevant source of the information and surrogate for assessing exposure to children as it would be a conservative estimate of what is likely to be higher exposure in children (ages 1 to 2) given the added dietary exposures.

Question 2 - Uncertainties with Using Biomarker Data from CCCEH for the PoD (Section 7.1)
Section 7.1 describes the key uncertainties in using the cord blood biomonitoring data from CCCEH as the PoD. While biomarker data are arguably superior to conventional exposure data in that they reflect chemicals that were absorbed in the body from all routes and sources, they do not provide direct measure of environmental exposure levels.

Chlorpyrifos in blood represents a snapshot of the concentration at a particular point in time. Uncertainty also exists when establishing a quantitative relationship between chlorpyrifos concentrations in blood and adverse health outcomes. For neurodevelopmental effects investigated in these epidemiologic studies, the adverse outcome pathways, toxic moieties, and biological targets were all unknown. The key assumption is that measured biomarker levels reflect exposures during time windows that were critical for disease onset. It is also not clear whether cord blood concentrations measured at birth reflect exposure levels during the critical time window(s). However, there is a reasonable likelihood that chlorpyrifos was applied multiple times in the apartments of the women in the cohort over the course of the pregnancy (potentially once a month) increasing the potential for exposure during those unknown critical period(s). In addition, in the context of the uncertainties associated with using the CCCEH blood data in quantitative risk assessment, there is concern that the PoDs based on AChE inhibition (Appendix 1) may not be adequately protective of human health. For example, given an external dose required to achieve 10% AChE inhibition for a female worker who was exposed dermally to chlorpyrifos 8 hours/day, 5 days/week for 3 weeks, the blood concentration of chlorpyrifos peaked at 120,000 pg/g, and was still above 100 pg/g at 32 days after the last exposure. Similarly, at a food exposure level leading to 10% AChE inhibition, chlorpyrifos concentration in blood never goes below 100 pg/g over the continuous 21-day exposure simulation and is around 7000 pg/g at the daily peaks.

Please comment on the Agency's characterization of the uncertainty associated with using the CCCEH blood data in quantitative risk assessment.

Panel Summary

The Panel agrees with the Agency that there are complex unknown variables that are recognized as uncertainties. Because many uncertainties cannot be clarified, the majority of the Panel does not have confidence that the CCCEH cord blood data on chlorpyrifos levels can accurately be used in quantitative risk assessment to determine a Point of Departure (PoD). The cord blood data were obtained from a single measurement at the time of delivery and the quantitative risk assessment made multiple assumptions based on this value. Some members of the Panel do not have confidence that a single value obtained at birth can be used to accurately estimate the magnitude or pattern of exposure that occurred during the preceding ten months of pregnancy. Some members of the Panel were also concerned about the lack of knowledge of the sensitive window(s) of exposure during pregnancy that would lead to neurodevelopmental outcomes. Without accurate knowledge that exposure occurred during a sensitive window, it is impossible to derive causation. A major source of uncertainty for the Panel was the lack of verification and replication of the analytical chemistry results that reported very low levels of chlorpyrifos (pg/g). Imputing quantitative values when the concentration of analyte falls below the level of detection (LOD) was a particular concern, especially given that a large fraction of cord blood samples included in the analyses presented with levels below LOD. While this may be appropriate in some epidemiological studies, some members of the Panel caution the Agency when attempting to use analytical data derived without Good Laboratory Practices (GLP) or chain of custody protocols in place for regulatory decision-making, while at least one Panel member found no issue with sample tracking and shipment reported in the CCCEH study. The Panel is not aware of any scientific

evidence where pg/g levels in the blood would lead to deleterious neurotoxicological effects in a mammalian system. This lack of data could indicate a lack of biological plausibility. The assumption that the impaired working memory and lower IQ measures observed are caused primarily by a single insecticide (chlorpyrifos) and predicted by the blood levels at time of delivery is not supported by the scientific weight of evidence. However, at least one Panel member noted that previous SAPs have evaluated the weight the evidence and have concluded otherwise.

Question 3 - Pharmacokinetic (PK) Time Course: Considerations for Labor & Delivery (Section 5)

Figure 2a-b (pg. 17-18) provides an example PK profile for chlorpyrifos for current exposures to pesticide applicators. Similar figures for food, water, and residential exposures are shown throughout the Agency's Chlorpyrifos Issue Paper (Sections 6 and 9). As shown in Figure 2a, each PK profile shows a consistent pattern of a daily, rapid increase in internal dose during the exposure period followed by a rapid decline after the exposure period ends. The rapid decline of chlorpyrifos after exposure terminates is expected given how rapidly chlorpyrifos is initially metabolized. The periods of rapid increase represent rapid uptake during activities that lead to chlorpyrifos exposures, while the periods of rapid decrease are primarily attributed to distribution from the central compartment (circulation) into the peripheral compartments (body tissues), loss to metabolism, and binding to esterase. For chlorpyrifos, the half-life of this initial phase is estimated to be approximately four hours. Upon cessation of the exposure, the terminal half-life (approximately 120 hours) predominates resulting in an asymptotic appearance for the internal dosimetry.

As summarized in Section 5, for deriving the proposed PoDs (Section 7), the Agency is assuming the CCCEH levels do not represent values within the rapid increase/decrease phase. Instead, the Agency is assuming the reported values for cord blood and maternal blood are at the low points or within the terminal clearance period (and thus unlikely changing significantly across several days). Although part of labor is spent at home (where exposure is assumed to occur), some portion was spent in the hospital—meaning removal from the apartment caused the exposure to cease. This assumption is being made because labor and delivery typically requires multiple hours. Moreover, maternal blood samples for some mothers were taken up to two days after delivery. The Agency also notes that the results from the Agency's exposure characterization analysis of the CCCEH (Section 6) closely match those from the CCCEH study, providing further support for the Agency's characterization of the PK profile.

> ***Please comment on the Agency's characterization of the PK profile and interpretation of the CCCEH biomonitoring data. Please include in your comments the Agency's proposal to use the 10 hour and 24 hour post-peak time points on the PK profiles for assessing risk to chlorpyrifos.***

<u>Panel Summary</u>

Multiple Panel members noted that PBPK modeling is a valuable tool to interpret the biomonitoring data in circumstances where multiple routes of exposure occur and when based on best available information as inputs. Concern was raised, however, by at least two Panel members about the following four points: 1) use of cord blood at delivery in

the CCCEH study as a point of departure (PoD) (rather than a simulated time-weighted average concentration during pregnancy, a peak concentration earlier in pregnancy, or even blood concentration at exit from home residence); 2) the assertion that cord blood measurements in CCCEH can be characterized as predominantly corresponding to levels 10-24 hours post peak; 3) a lack of justification of an absence of chlorpyrifos exposure between admission and the collection of cord and maternal blood; and 4) the absence of a sensitivity analysis that would help characterize the dependence of key model outputs on particular parameters. Panelists differed in their responses as to the level of agreement between the Agency's exposure characterization of the CCCEH and the blood measurements from the study, with one Panel member noting that the Agency's phrase "closely match" is highly subject to interpretation. One Panel member noted that the characterization of the PK profile appropriately uses and interprets the CCCEH biomonitoring data and that the exposure characterization provided by the Agency in Section 6 closely matches the real human data that the CCCEH study has to offer.

Question 4 - Evaluation of CCCEH Cord Blood Data & Predicted Exposures to the Cohort (Section 6)

The Agency has used the PBPK model to predict blood levels in women across several exposure scenarios for comparison with the cord blood levels reported by the CCCEH (Section 6). Food exposure is expected to have occurred (Section 6.2), whereas drinking water exposure was unlikely (Section 6.1). Given the lack of specific CCCEH exposure information, the Agency has developed six possible residential exposure scenarios representing a broad range of residential post-application exposures to chlorpyrifos products available prior to the voluntary cancellation of indoor products in 2000 (Section 6.3). Two exposure scenarios were conducted using USEPA standard residential exposure assessment approaches; these two scenarios represent the high end exposure potential. To estimate lower exposures, four additional PBPK model simulations were conducted with use of reported values from the CCCEH investigators. These six possible residential exposure scenarios were input into the PBPK model to predict a range of potential exposures for comparison to the predicted internal dosimetry levels reported by the CCCEH investigators. Based on the results of these simulations, the Agency has concluded that: 1) the reported higher blood levels in the CCCEH from 1998-2000 are likely driven primarily by residential use of the broadcast and perimeter chlorpyrifos products registered for use at that time; and 2) these results further support the reasonableness of the magnitude and distribution of data reported by CCCEH.

> ***Please comment on the Agency's conclusions that these scenarios adequately capture the range of exposure. Please also comment on the Agency's simulations from residential and food exposures and the degree to which the estimates of internal blood levels do or do not match the CCCEH cohort results before and after the cancellation of indoor products in 2000.***

<u>Panel Summary</u>

Overall, the Panel found that the general scenarios provided for PBPK modeling are reasonable (drinking water, food, residential). There is some uncertainty in the drinking water data related to sample timing and frequency. The contribution of food is probably relatively small but not completely absent. With regard to the residential chlorpyrifos

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exposure scenarios, they generally capture the kinds of exposures one would expect in the residential setting but again, uncertainty lies in the timing and frequency of residential exposures relative to the timing of cord blood collection. The Panel found several sources of uncertainty in the estimates of internal blood levels and their relationship to the CCCEH cohort results. One of the sources of uncertainty is the use of a single point estimate in the rapid-decline phase of the simulation, which raised the question of whether an "area under the curve approach" might better address the inherent variability of potential exposures likely to occur in the residential setting. Two important sources of uncertainty are variability of the cord blood samples within the CCCEH study and the fact that they were likely collected during the terminal clearance phase, which does not accurately predict overall gestational exposure. The Panel also noted that the exclusion of the time period after the ban of residential use may make the PBPK model more conservative. Finally, some Panel members thought the quality of the CCCEH data is hard to assess when raw analytical data have not been made available, and the study has not been reproduced. These factors contribute to the overall level of uncertainty. One Panel member did not find these points to be limitations.

Question 5 - Options for Deriving a PoD for Neurodevelopmental Outcomes Based on the CCCEH Biomonitoring Data (Section 7.2)

As summarized in Section 7.2, the Agency has proposed a PoD for the observed neurodevelopmental effects and offered alternative options based on internal blood concentrations of chlorpyrifos from the results of the Columbia University study.

> **Question 5.a. Approach to Using the Cord Blood** - The Agency could consider continuing to use the AChE PoDs and apply additional factors over and above the Food Quality Protection Act (FQPA) 10X Safety Factor to reflect the level of uncertainty of protecting for neurodevelopmental outcomes when using AChE for the PoD. However, the Agency would still need to quantify the difference between effects from AChE inhibition and from neurodevelopmental outcomes—and the analysis to evaluate the appropriate additional factor(s) would again require the Agency to make quantitative use of the CCCEH cord blood data with the same uncertainties described above. The Agency has elected to propose to use the cord blood directly as the PoD as the simpler, more understandable approach. ***Please comment on the Agency proposal to use cord blood directly as the PoD.***

Panel Summary

The Panel agrees that both epidemiology and toxicology studies suggest there is evidence for adverse health outcomes associated with chlorpyrifos exposures below levels that result in 10% RBC AChE inhibition (i.e., toxicity at lower doses). The Panel also agrees with the Agency in that applying additional safety factors to the AChE PoDs to account for a possible noncholinergic MOA would be problematic because of challenges in justifying any particular value for such an adjustment. While one Panel member agrees with the Agency's simpler approach of using the CCCEH study cord blood data for directly deriving the PoD, the majority of the Panel considers the Agency's use of the results from a single longitudinal study to make a decision with immense ramifications based on the use of cord blood measures of chlorpyrifos as a PoD for risk assessment as premature and possibly inappropriate. The basis for this majority view includes: 1) an

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inability to either know, or confidently make assumptions about, aspects of exposure patterns, labor and delivery, and blood collection; 2) uncertainty in the use of the PBPK model for extrapolating the chlorpyrifos exposure concentration using the cord blood measures; 3) concerns with transparency given that the neurobehavioral data matching the four highest measured cord blood values were not included in the working memory analysis; 4) uncertainty in the timing of the biomarker measurements related to developmental window of susceptibility; 5) lack of biological plausibility for how low cord blood (low parts per trillion) concentrations of chlorpyrifos can alter working memory and produce neurodevelopmental impairment; and 6) issues with the estimation of missing cord blood values using maternal blood values. One Panel member indicated that the most important limitation with relying on the available information is that it is only a single study (*e.g.*, CCCEH).

> **Question 5.b. PoD Options** – From the CCCEH publications, there are two general options that EPA has considered for deriving a PoD for extrapolating risk to chlorpyrifos: 1) Lower limit of the top tertile (>6.17 pg/g cord blood) derived from Rauh et al (2006) and repeated in other CCCEH publications; or 2) Benchmark Dose (BMD) estimates derived from linear regression reported in Rauh et al (2011) for deficits in Working Memory. Rauh et al (2011) reported that for each standard deviation increase in exposure (4.61 pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory. The Agency has decided to use the BMD approach.
> ***Please comment on the PoD options considered by Agency.***

Panel Summary

The Panel was not in favor of using the lower limit of the top tertile for deriving a PoD for extrapolating risk to chlorpyrifos (Option 1). The Panel was not satisfied with the arbitrary cut-off for the tertile distributions, the high-low dichotomization in the analysis, and the differences in interpretation based on the high-low dichotomization compared to that obtained when grouped into a ND, low, mid, and high groups. With respect to Option 2, the Panel considers the Agency's decision to use BMD derived from linear regression for deficits in working memory as a valid plan if the Agency were to decide to use cord blood chlorpyrifos to determine PoD. However, with the exception of one Panel member, the Panel stated that using cord blood chlorpyrifos concentrations for derivation of the PoD could not be justified by any sound scientific evaluation. The BMD for IQ and working memory is directly linked to the cord blood concentrations and, as expressed in previous responses, there are multiple uncertainties associated with the cord blood data. Some members of the Panel also expressed concern that there were multiple chemicals, including many neurotoxic pesticides, present in the households during pregnancy. Several of these pesticides have the same mechanism of action as chlorpyrifos, suggesting that simultaneous exposure with chlorpyrifos could result in an additive effect, yet the PBPK models presented account for chlorpyrifos as a single isolated exposure. One Panel member noted the efforts of the researchers to adjust for a reasonable set of co-occurring exposures to show chlorpyrifos to be the pesticide driving the adverse outcome.

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Question 5.c. Agency's Proposal for PoD – The Agency proposal applies the BMD approach to the Rauh et al (2011) study, and the Agency has selected a 2% change in working memory or an internal dose of 2.16 pg/g as the PoD. This Agency proposed value is quantitatively near the value reported by Rauh (2.8% reduction in working memory) and thus supported by the existing data, but is still health protective and conservative.
Please comment on the analysis/calculations used to derive these estimates as described in Appendix 6 and the selection of a 2% response level.

Panel Summary
The Panel differed with respect to the importance of a 2% change in working memory. Many Panel members considered a 2% change in working memory (less than one standard deviation in the distribution of scores in the general population) to be of questionable biological significance. Other Panel members stated that a 2% shift in such a parameter would increase the prevalence of intellectual disability in that population. While several Panel members found the analyses/calculations to be appropriate, the majority of the Panel agreed that the Agency provided insufficient justification to utilize this methodology for the purpose identified in the Agency's presentation. The issues of relevance and biological plausibility were especially problematic given that home use of chlorpyrifos has been discontinued since 2000.

Question 6 - Assessing Extrapolation/Uncertainty (Section 8)
In typical risk assessments, PoDs are derived directly from laboratory animal studies and inter- and intra-species extrapolation is accomplished by the use of default 10X factors. In the case of chlorpyrifos, the proposed PoDs are derived from human information obviating the need for the inter-species extrapolation. However, the Agency still needs to consider intraspecies extrapolation of the PoD from the CCCEH epidemiology data across the diverse human population (Section 8.1). Moreover, the Agency must consider the statutory requirement of the FQPA 10X Safety Factor for "potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children" (Section 8.2).

Question 6.a. Intra-species extrapolation – For chlorpyrifos, the Agency proposed to use a 10X intra-species extrapolation factor. This 10X, apportioned equally between 3X for PK variability and 3X for PD variability is consistent with that used previously by the EPA IRIS program for methyl mercury (Appendix 8).
Please comment on the Agency's scientific rationale of the proposed use of a 10X intraspecies extrapolation factor.

Panel Summary
Overall, the Panel found that the use of the 10X intra-species extrapolation factor is appropriate; however, there are other areas of uncertainty that should be considered. Uncertainty is mostly attributed to the inability of single measures of chlorpyrifos concentration in blood to provide information regarding source, frequency, duration and magnitude of exposure, and how these exposures are linked to specific outcomes measured in the CCCEH study participants. The degree to which the CCCEH study population is predictive of the US population in general is also unknown. Finally, with

the use of a PoD that provides a 10X dose that is below the Limits of Detection (LoD), it may be impossible to regulate on levels that cannot be measured.

> **Question 6.b. Pre- vs. Post-natal Exposure** – Numerous epidemiological investigations have observed a link between prenatal exposure to chlorpyrifos or OPs and adverse effects on neurodevelopment through age seven years, with additional more limited evidence up through approximately age eleven years. By contrast, epidemiological evidence is more limited for associations between postnatal exposure to chlorpyrifos or other OPs and neurodevelopmental effects, and the CCCEH study has specifically not assessed these associations. Therefore, given that the extensive experimental laboratory animal database suggests that the postnatal period is a potential susceptible time, the lack of postnatal exposure assessment in the CCCEH and other similar cohort studies is a source of uncertainty in the epidemiology database.
> ***Please comment on the Agency's conclusion that the lack of postnatal exposure assessment in the CCCEH study is a source of uncertainty in the epidemiology database.***

Panel Summary

Overall, the Panel concurs with the Agency's conclusion that the lack of postnatal exposure assessment in the CCCEH study is a source of uncertainty. While the prenatal period is likely the most sensitive to chlorpyrifos exposure, the brain continues rapid development postnatally. Neurogenesis, synaptogenesis, myelination, etc. continues in early life and brain development proceeds into adulthood. Working memory is an important component of executive function and is the primary endpoint associated with chlorpyrifos exposure in the CCCEH study. One Panel member noted that since the primary source of exposure was removed early in the CCCEH study (ban on indoor use), exposures occurring postnatally in the CCCEH birth cohort would have been significantly reduced, and therefore the CCCEH study provides little information on the effects of postnatal exposures on neurodevelopmental health outcomes.

> **Question 6.c. Impact of Sample Size on CCCEH Findings** – Associations with neurodevelopmental outcomes were consistently identified with respect to the number of abnormal reflexes in the neonatal period, the presence of mental and behavioral issues as well as gross motor delays were pronounced especially in at ages 24-36 months, and the observation of intelligence decrements at age seven years were seen across the three US childrens cohorts using different measures of prenatal chlorpyrifos exposure. However, with regards to dose-response, the modest sample size in the CCCEH study make it difficult to say that the dose-response relationship between exposure to chlorpyrifos and neurodevelopmental outcomes in the overall U.S. population has been fully characterized. The magnitude of the PoD in the general U.S. population of infants and children may be higher or lower than that estimated using the CCCEH study results, and the shape of the dose-response curve may also be different.

Please comment on the Agency's conclusion that the moderate sample size of the CCCEH study is a source of uncertainty, given that the Agency is proposing to use the CCCEH study data directly for setting a PoD.

Panel Summary

The majority of Panel members stated that the moderate sample size of the CCCEH is not a source of uncertainty, however some Panel members did identify uncertainties. Two Panel members generated a power curve based on a sample size of 265 participants and the results indicate the CCCEH study had the ability to detect reductions in Working Memory Index as small as 0.1 per 1 pg/g increase in chlorpyrifos blood concentration, which is much lower than the reductions reported in Rauh *et al.*, 2011. Thus, the sample size should not have impacted the study's ability to detect associations of chlorpyrifos blood concentration on neurodevelopment, and more specifically, subscales of the WISC-IV. Some Panel members noted that the sample size may have limited the CCCEH study's ability to examine the association of chlorpyrifos blood concentration on neurodevelopment in more vulnerable populations, such as racial minorities or lower socioeconomic status individuals. The small sample size also limited the extrapolation of the findings to the U.S. population in general, and adds to the difficulty of analyzing samples at or near the LoD. One Panel member noted that the issue involves not overall sample size, but the use of a small subset of values for various studies. For example, the use of 34% of a sample to measure blood lead levels (Rauh *et al.*, 2011), and the use of between 4-5 % of a sample of children in relating high chlorpyrifos exposure and attention problems (Rauh *et al.*, 2006).

Question 7 - Proposed Approach to Deriving Internal Dose Estimates: Integration of Exposure Assessment & PBPK Modeling (Section 9)

The Agency has proposed to input exposure estimates for chlorpyrifos into the PBPK model to assess internal blood concentrations from current exposure patterns. Several case examples were provided in the draft issue paper representing food exposures (Section 9.2), drinking water (Section 9.3), and worker exposure (Section 9.4). [Note: Exposure assumptions used in these examples have been previously reviewed by other SAPs.]

Please comment on the implementation of the PBPK model using such exposure inputs and interpretation of respective simulated blood levels.

Panel Summary

The Panel decided the model was appropriately implemented with regard to dose linearity and the duration of the simulations. The simulated blood concentrations were in the pg/mL range, so saturation of metabolism was not a concern. Other model parameter values such as tissue / blood partition coefficients and blood flows to tissues were unlikely to be affected by the relatively low chlorpyrifos blood concentration, so the assumption that internal concentration would be linearly related to external dose rate was appropriate. Simulations of the various exposure scenarios were run well into the steady-state time frame. The inputs of chlorpyrifos to the PBPK model from drinking water, food, and worker – handler exposure appeared to be appropriate and defensible. The inputs were well informed by a considerable amount of data relating to expected drinking

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water concentrations, and dietary contributions. Worker – handler inputs were informed by highly developed occupational exposure methodologies and exposure data sources that have been extensively peer reviewed. There were no compelling issues with regard to the suitability of the inputs. Of the three exposure modalities, the Worker – Handler scenario produced the highest chlorpyrifos blood concentrations: the average peak concentration for seven scenarios was 393 pg/g blood (range 194 – 954), the average concentration after 24 hr was 5.99 pg/g (range 3.0 – 12.4), and the average concentration at 10-hr-after-last-peak on Day 12 was 31.9 pg/g (range 16.0 – 71). For the food scenarios venous blood concentrations were much lower than for the worker – handlers with the average peak concentration during the 120 d simulation being 0.67 pg/g blood at the 50th percentile and 7.14 pg/g at the 99.9^{th} percentile. Average peak blood concentrations for the drinking water scenarios were similar at 7 pg/g for the onion crop scenario and 2 pg/g for the Orestimba Creek scenario. These simulated concentrations were well above the proposed Reference Dose (RfD) of 0.022 pg/g internal concentration. If the simulated internal chlorpyrifos concentrations and the proposed RfD were realistic, large adverse effects should be seen in babies born to women who are worker – handlers of chlorpyrifos or who experience the food-borne chlorpyrifos input scenario. The food exposure scenario suggested that almost 90 percent of the adult female population always would have blood chlorpyrifos concentrations above the proposed RfD.

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DETAILED PANEL DELIBERATIONS AND RESPONSE TO CHARGE

Question 1 – Lifestages for Consideration (Section 4)
Fetuses may be exposed to chlorpyrifos through the mother, while infants and children are exposed directly through dietary exposure (food and drinking water). The Agency has conducted case studies to use cord blood data as a surrogate to evaluate the health impacts on fetuses and infants from exposures to chlorpyrifos (Section 6). Based on the available data, there are several assumptions that are being made in order to assess exposure for specific lifestages.

> **Question 1a. Fetal exposure** – Without a gestational model that is parameterized with chlorpyrifos information, fetal exposure cannot be assessed directly. However, maternal and cord blood concentrations of chlorpyrifos from CCCEH are highly correlated (Figure 1), and preliminary evaluation of the Dow gestational PBPK model suggests little differences in blood levels between pregnant and non-pregnant women. Therefore, the Agency has concluded that the parameterized model which is available for females (13-49 years old) can be used as a reasonable surrogate for estimating fetal exposure.
> ***Please comment on the Agency's proposal to use female blood levels as a surrogate for fetal exposure.***

Panel Response
The Panel supports the use of female blood levels as a surrogate for fetal exposure. The chlorpyrifos levels measured in fetal cord blood correlate well with the mother's chlorpyrifos blood levels. Specifically, the fetal cord blood concentrations track the mother's blood concentrations. This suggests that chlorpyrifos readily crosses the placenta, thus the fetal cord blood and the rest of fetal systemic circulation is assumed to track temporal fluctuations in the mother's blood. However, one Panel member contends that Figure 1 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) is not statistical evidence as Spearman rank correlations are only qualitative. This Panelist also pointed out the limitations of the evidence and the issues with the cord blood concentrations introduced by the sampling protocol and duration of labor (see area of uncertainty #3 below).

Whyatt *et al.* (2003) showed that chlorpyrifos concentrations in maternal and umbilical cord plasma has a Spearman rank correlation of 0.76 in n=180 mother-child pairs. Then in 2009, Whyatt *et al.* used the same nonparametric Spearman rank tests to show that chlorpyrifos concentrations in maternal and umbilical cord plasma were highly correlated $r = 0.9$, $p<0.001$, in $n = 64$ mother-child pairs. These high correlation coefficients suggest that tracking the blood concentrations of the mother is a reasonable surrogate for the corresponding levels in fetus.

However, there are several areas of uncertainty that the Panel identified as needing clarification.
1) The key study uses imprecise terminology, with "blood levels" and "plasma levels" used interchangeably, yet they do not mean the same thing. Whyatt *et al.* (2003), in the methods section, clearly described the collection of blood into anticoagulated tubes, which were centrifuged to collect plasma that was then sent for analysis of pesticides

including chlorpyrifos. But in some tables of the Whyatt *et al.* (2003) paper, the data are reported as plasma concentrations while in other tables as blood concentrations, when plasma concentrations seem in fact to have been measured and listed. Figure 1 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) presents chlorpyrifos "blood level" data from Columbia Center for Children's Environmental Health Mothers and Newborn Study (CCCEH), but these actually appear to be plasma concentrations. This imprecision has implications for the physiologically based pharmacokinetic (PBPK) modeling as the model is apparently parameterized using blood volumes and flows and tissue/blood partition coefficients. The model-simulated blood concentrations then seem to be compared with observed plasma concentrations. This needs to be sorted out and clarified as does the possible use of chlorpyrifos binding to formed elements (red blood cells (RBCs) and platelets). If blood is to be the reference fluid, then tissue/blood distribution rather than tissue/plasma distribution coefficients may be more appropriate. A complication is that upon tissue uptake of unbound chlorpyrifos from plasma, the plasma-protein-bound chlorpyrifos would likely dissociate and become available for extraction during passage of plasma through a tissue capillary bed. Dissociation of chlorpyrifos bound to formed elements would generally be much slower than from soluble proteins and during tissue transit may or may not be released and become available for tissue uptake.

Another study that utilized imprecise terminology regarding the interchangeable use of "blood" and "plasma" concentrations is Timchalk *et al.* (2002). Figure 3 of Timchalk *et al.* (2002) presented chlorpyrifos blood concentrations (rat), whereas in Figure 8 (human) plasma concentrations were presented. The methods section indicates that the same analytical method was used, and the referenced method (Brzak *et al.*, 1998) indicates that whole blood was analyzed for chlorpyrifos and chlorpyrifos-oxon (CPFO). Hattis *et al.* (2011; p. 70 Preliminary Comments) also points out this problem in the context of lipid content of plasma vs. blood.

2) The percentiles of the chlorpyrifos concentrations in cord plasma at delivery and in the mother's plasma at or shortly after delivery for "all years" in Figure 1 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) appear to have consistent cord-to-mother concentration ratios approximating 1.0 – 1.1 across percentiles when data from 1998-2004 are combined. However, with respect to data for individual years beginning at 2001, the majority of points for both fetal and maternal samples are below the level of detection, which adds uncertainly to the information. The apparent central tendency for the mother's concentration to be slightly lower than the cord concentration may be attributed to differences in the time elapsed between collection of the maternal and cord samples. Moreover, Hattis *et al.* (2011; p. 41) discusses blood lipid differences between cord and mother's blood that may also contribute to quantitative differences. Relative to the cord sample collection, collection of mother's plasma samples was delayed as much as two days, although with a terminal phase half-life of 120 hours, the mother's plasma concentration would not decline much if the delay were relatively small. For example, chlorpyrifos concentration would decline by about 10% after 18 hr. It therefore appears that under steady-state conditions (pseudo-equilibrium of chlorpyrifos between mother's and cord plasma) the chlorpyrifos concentrations in mother's plasma and cord plasma

could be approximately equal, and the mother's plasma chlorpyrifos concentration would be an excellent surrogate measure of the cord plasma concentration and therefore an excellent surrogate for exposure of the fetus to chlorpyrifos at the time that the mother's blood was sampled. Given the model-predicted half-life of 120 hr for chlorpyrifos and assuming steady-state conditions, the mother's blood concentration should be a reliable surrogate for the fetal blood concentration for two half-lives on either side of the time of sample collection; *i.e.*, 10 days before to 10 days after the sample collection. This assumes that the PBPK model-predicted half-life is operative; supporting validation data are generally lacking although one poisoning incident provided a chlorpyrifos serum concentration-time profile for 15 days (Timchalk *et al.*, 2002; Fig. 11), with the final 11 days clearly supporting the 5-day half-life predicted by the model. (The half-life in the publication that presented the poisoning case, for the particular individual was 4.1 days.)

For the cord plasma and mother's plasma concentrations to be equal at steady state,

- the permeability of the placental barrier chlorpyrifos would have to be relatively high such that a change in mother's plasma concentration over time would be tracked closely by a similar change in the cord plasma concentration.
- the extent of binding of chlorpyrifos to plasma proteins would have to be the same. This would support an assumption that the free (unbound) toxicologically active chlorpyrifos concentrations were similar in both mother's and cord plasma.
- net active transport of chlorpyrifos across the placental membranes, if it occurs, would have to be small relative to passive transport so that a concentration gradient between mother's and cord plasma did not exist at steady state.

The PBPK model for chlorpyrifos and chlorpyrifos-oxon (CPFO) has been extended to include uterine, placental and fetal compartments (Poet, 2015). This model could be used to simulate chlorpyrifos and CPFO concentrations (free and total) in mother's and cord plasma, to investigate the closeness of the concentration-time (C,t) profiles after various inputs of chlorpyrifos to the mother and to investigate the degree of correlation among mother's plasma, cord plasma and fetal CNS concentrations of chlorpyrifos and CPFO. The temporal linkage of the model-predicted C,t profiles would help to gauge how well mother's plasma reflected the toxicologically active concentrations in the fetal target tissue.

The blood concentrations under consideration are only applicable for the last exposure(s) to chlorpyrifos prior to birth. This period would only encompass approximately 30 days at maximum based on the PBPK modeling of various exposure situations. There are uncertainties: the exact timing of the exposure(s) is unknown, and several scenarios are possible. For example, a low level application of chlorpyrifos immediately prior to labor would result in values that have not fully reached terminal half-life. Alternatively, exposure to a very high application of chlorpyrifos made earlier than the suggested 30 days could lead to different conclusions. The exposure model was created assuming that the mother would only spend a certain specified amount of time in proximity to chlorpyrifos residues. However, during the 30-day simulation, the expectant mother in the final stages of gestation would likely be less mobile and thereby spend more time in contaminated areas of the home, which would delay the rapid initial drop in chlorpyrifos

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levels predicted by the PBPK simulation. Using a single value as a marker for exposure during the entire period of pregnancy does not seem to be an accurate assessment (oversimplification) of exposure. This point was highlighted by a slide shown by the Agency in which four different exposure scenarios resulted in the same measured blood level at a single time point. The measured blood concentrations cannot detect any exposure to chlorpyrifos occurring earlier during the pregnancy, and are unlikely to reflect exposure throughout the prenatal period, important factors that are likely to influence biologically plausible outcomes.

3) Arguments using levels in females as a surrogate for fetal exposure have limitations that could be clarified with access to more detailed information from the researchers conducting the CCCEH study. The data in Figure 1 of the Agency Chlorpyrifos Issue Paper (USEPA, 2016) are generally supportive of the proposed practice. However, the available evidence for correlation is limited to data based on discrete percentile levels, and not on actual paired data. In some years, the numbers of cord and maternal samples are similar (110 vs. 120, in 2000), but not in other years (138 vs. 72 in 1998-1999). Even when the total numbers are similar, the extent to which these data represent pairs of mothers and newborns is unknown (i.e. in 1998-99 there could be maternal samples with no corresponding cord sample, so there could be fewer than 72 matched pairs), but there are at least 66 cord samples with no corresponding maternal sample. Thus, even if the maternal:cord concentration ratios are similar at the various percentiles and across years, these ratios may not be representative of paired maternal:cord samples. The 1998-99 samples could even be interpreted as indicating lack of cord vs. maternal similarity, at least at lower exposures. For instance, the 25th percentile cord blood sample for 1998-99 (n= 138 samples) was below limit of detection (*i.e.*,<0.500 pg/g), whereas in contrast, the 25th percentile maternal sample for the same period (n= 72) was 2.6 pg/g, 5-fold greater than the limit of detection (LOD). At the 10th percentile in the same year, the maternal level of 1.5 pg/g was 3-fold greater than the LOD. These greater maternal levels are observed despite potential delays in sample collection ('within 2 days postpartum' and not 'as close to delivery as possible' like the cord blood", page 20). Where detectable levels are available, the maternal blood concentration/cord blood concentration ratios at the various percentiles range from 0.8 to 1.8; a rough estimate of the central tendency for the maternal/cord blood concentration ratio of ~1.3 can be derived from these data. This pattern is in fact, consistent with the formula developed by Whyatt *et al.* (2004) and used by Rauh *et al.* (2011) for imputing the values of missing cord blood samples from maternal blood data (described further herein in response to Charge Question 5a) that also indicates maternal blood values can be higher than cord blood values.

A better approach would be to derive maternal/cord ratios from original paired data rather than a limited number of unpaired values at discrete percentiles. Hattis *et al.* (2014) reports doing just such an analysis for 191 records where both samples were available. The geometric mean was 1.2, with a 5th-95th percentile range of 1.065 to 1.35.

4) Both maternal and fetal biomarkers have some of the same weaknesses. The most serious weakness is the fact that each measurement is only collected once during the pregnancy, that one time is at the end of gestation, and the end of gestation is probably

not the critical prenatal period for induction of neurodevelopmental effects. For instance, Appendix 3 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) reviews numerous animal studies in the literature that provide support for the susceptibility of the developing mammalian brain to chlorpyrifos exposure throughout gestation and early in life. "Overall, these data do not clearly show specific critical periods of exposure but support a conclusion that early life (pre- and post-natal) represent susceptible lifestages." (USEPA, 2016; p. 47). Thus, it is difficult and perhaps indefensible to extrapolate either blood value to exposures that may have occurred months prior to sampling.

5) Both maternal and cord blood are expected to have short-term and long-term peaks. The Agency Chlorpyrifos Issue Paper (USEPA, 2016) contained numerous examples of peak levels of maternal blood calculated by the PBPK model; however, no model predictions of peak levels of cord blood were provided to the Panel. Although there is evidence that chlorpyrifos in maternal blood is often similar to that in cord blood (USEPA, 2016; p. 13, 17, 138), there is also evidence to indicate that under certain exposure/sampling paradigms they are very different (see responses to Charge Questions 3.a. and 5).

> **Question 1.b. Infant (<1 year old) exposure** – Studies of chlorpyrifos in laboratory animals do not suggest any specific critical period or lifestage, but instead suggest both pre- and post-natal periods of susceptibility. In contrast, there are limited epidemiological evidence regarding postnatal exposure to chlorpyrifos or other OPs to infants and children. Because brain development continues through childhood, and due to the concern that AChE inhibition may not be protective of the neurodevelopment outcomes, the Agency is proposing to use the chlorpyrifos cord blood data from CCCEH as the most relevant source of information for deriving a PoD for infants (see Appendix 4.0 for further details). ***Please comment on the Agency's proposal to use cord blood data as a surrogate for assessing infant exposure.***

Panel Response

Most of the Panel was not in favor of using cord blood as a surrogate for the infant. It is noted that it is possible that additional chlorpyrifos exposure in the postnatal period may have occurred. Thus, this added exposure also has the potential to negatively impact neurological development in addition to prenatal exposure. Using the CCCEH study information on chlorpyrifos cord blood would be a useful and relevant source of the information for deriving a PoD, as it would be a conservative estimate of what is likely to be higher exposure in infants. However, one Panel member stated that the fetal cord blood samples can be used as a PoD.

The cord plasma concentrations of chlorpyrifos and chlorpyrifos-oxon (CPFO) are likely an accurate reflection of the infant's exposure at the time of birth. As time passes, the infant plasma concentrations (chlorpyrifos, CPFO) would follow a path that was set by the rate of input to the infant's systemic circulation and the pharmacokinetics (PK) of the substances in the neonate. During the initial few half-lives of chlorpyrifos and CPFO, exposure of the infant would less and less be reflected by the cord plasma concentrations,

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and more and more by the recent dosing history and operative PK in the infant. Based on a floor treatment residue dissipation rate of 10% per day (USEPA, 2016; p. 29) being mathematically equivalent to a half-life of 6.6 days, two half-lives would be 13 days, three would be 20 days, and four 26 days. Exposure could be increased, decreased or unchanged from that at birth.

It seems reasonable to expect that the mother's home environment and diet, which led to the cord plasma chlorpyrifos concentration would remain the same once the infant subsequently returned to the dwelling. But it would also be expected that quantitative differences in the intake and PK in the infant compared with the mother prenatally would lead to a different systemic chlorpyrifos exposure than that indicated by cord plasma data.

The PBPK life stage model could be used to project the infant's exposure over time post-birth, using cord plasma concentration and an assumption of steady state to set initial conditions for the tissue compartments. With no further chlorpyrifos exposure, such a projection would indicate the time frame for the utility of cord plasma data to reflect exposure. Realistic dosing scenarios could be added to gain insight into the possible systemic exposure time course over the 0-1-year life stage. An exposure scenario based on the mother's plasma concentration could reasonably be used for the infant assuming that the home environment of the mother at birth would be similar to the one in which the infant was living.

If postnatal exposure to chlorpyrifos has no contribution to neurodevelopmental effects, measures related to pre-/peri-natal exposure would be expected to have greater relevance to potential risk for infants. In the event that postnatal exposure is relevant to the reported neurodevelopmental effects, to the extent that place of residence, household treatment protocols, and hygiene from the prenatal period are likely to carry over to the early postnatal period, it seems plausible to use cord blood (or predicted maternal levels) as a surrogate for infant exposure in the CCCEH study or other infant scenarios. However, measured cord blood should not be used directly as the best estimate of risk-relevant perinatal exposure; it should be adjusted to reflect possible peak or time weighted average (TWA) blood concentrations of chlorpyrifos (see answer to Charge Question 2) throughout pregnancy using the time for labor and delivery. Since the Agency has a young life-stage model (infant model) and has simulated infant exposure to chlorpyrifos, then the question is, "Can the cord blood chlorpyrifos measurements be used as a PoD for evaluating the infant exposure?" This assumes that an infant less than one year of age may suffer from the same adverse effects, as those found in children who were exposed in utero. There is substantial uncertainty in the window of susceptibility. Therefore, using cord blood measures only provides a 'bounding' exercise for exposure simulations. In the modeling work, it was unclear if the newborn contained the calculated masses of chlorpyrifos found in the fetus as birth. This body burden would slowly clear from the newborn, thus only for days after birth may the newborn exposure resemble the fetus exposure.

Timing is of the essence. Only for fetal exposure is there possibility to use the pregnant female blood levels as a surrogate for her fetal exposure, and even then, one would not

know if the levels were at a peak or trough of exposure. After birth, exposure patterns of mother and infant are no longer the same; exposure of the infant is totally separate from that of the mother with a potential common exposure pathway being via inhalation. The only exposure connection between infant and mother would be breastmilk, otherwise exposure will be through food, water and direct contact. As the infant becomes mobile (6-10 months) and spends more time crawling on the floor, dermal and potentially inhalation exposures may occur and diverge from those of the mother. These exposures are totally independent of the cord blood values. Thus, cord blood data cannot be used as a surrogate for assessing infant exposure for the entire first year post-birth.

There are, however, considerable questions about the blood data itself, as the original raw data have not been provided for review by the Agency or the Panel. Among the major issues, non-detect values appear to have been included in evaluations of mother-infant pairs. There is uncertainty as to whether cord blood is able to provide information about fetal exposure. Cord blood is likely to under- or over-estimate fetal exposure. While cord blood data may be directly relevant to fetal exposure levels, they may be less relevant to infants and children. There are important differences among the preterm neonate, term neonate, and young infant in terms of pharmacokinetic parameters which are influenced by variables such as gestational age, body composition, postnatal age, concomitant drug therapy, acidemia/hypoxemia, and end-organ perfusion.

The current model being used for chlorpyrifos risk assessment does not include gestational or lactational exposure. However, there are other models of lactation exposure available. While the modified model reasonably simulates the physiological changes during pregnancy, the model's predictive ability to simulate internal dosimetry of chlorpyrifos cannot be properly evaluated since there were no chlorpyrifos-specific pharmacokinetic data available during pregnancy.

Cord blood may be able to serve as a surrogate for the neonate, defined as children from birth to the age of 28-30 days of postnatal life (under some conditions), and which includes both preterm and term neonates. However, there are differences in absorption, distribution, metabolism and excretion (ADME) parameters between the newborn and the neonate which should be considered in using cord blood as a surrogate even for this age group (Tayman *et al.*, 2011).

The PBPK simulations within the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) show that the chlorpyrifos in the mother's blood from recent exposures is undergoing a rapid initial phase of elimination with a half-life on the order of 4 hours. The data in the review by Neal *et al.* (2010) show that among healthy, low-risk, nulliparous women at term with a spontaneous labor onset, the 'active phase' of labor lasted an average of 6.0 hours with a standard deviation of 3.5 hours. No information about the maternal parity of the CCCEH cohort has been provided, but the Agency's Chlorpyrifos Issue Paper (USEPA, 2016; p. 19) indicates that "Reported times for labor and delivery … may become faster for subsequent pregnancies." Because any given expectant mother's blood level caused by a recent exposure that creates a short term peak which in turn has the potential to decrease by a factor of 8x while she is in labor, the distribution (highs, lows,

range, and mean) of the values of chlorpyrifos in cord blood reported in Rauh *et al.* (2011) are likely to have been affected as much if not more by variations in the duration of labor (or time outside the home) than by either the initial (but unmeasured) levels of those peaks or the long-term slowly changing concentrations indicative of terminal clearance (as measured in maternal blood levels and as described in response to Charge Question 1.a.). While the effect of this phenomenon was not sufficient to prevent the regressions depicted in that figure from showing a significant correlation between cord blood levels and adverse Working Memory scores (and in fact, the point was made by several members of the Panel that misclassifications always dilutes the ability to find such associations), no analysis was made of the impact of this phenomenon on the precision of the slopes and the reverse accuracy of the predicting the chlorpyrifos necessary to cause a particular magnitude of neurodevelopmental outcome, and in turn establish a PoD.

Although this charge question is not asking for alternatives to cord blood, the Agency should consider investigating the use of maternal blood as the surrogate for assessing fetal exposure. Although maternal blood is one step further removed from the biologic target system (the cord and then the fetus), the timing of collecting the maternal blood sample while in the terminal phase assures that it is not being affected by recent exposures and short-term spikes.

> **Question 1.c. Children (ages 1<2 years old) exposure** – At this time, the Agency has not included a case study for evaluating the health impacts on children 1<2 years old. However, the Agency is aware that this age group often has the highest exposure from food consumption (Section 4), as is the case for some food commodities for chlorpyrifos exposure. Children 1<2 years old have not yet been included in the case studies as these ages are temporally removed from gestational exposure; as such the relevance of the cord blood data to predict the outcomes in toddlers is unclear.
> ***Please comment on the strength and uncertainties of using the CCCEH cord blood data as a surrogate for assessing (children ages 1<2 years old) exposure to chlorpyrifos.***

Panel Response

Most of the Panel was not in favor of using cord blood as a surrogate for the infant. Many of the answers given to Charge Question 1.b. also apply to 1.c. Given the ~5-day terminal half-life of chlorpyrifos, it would seem unreasonable to think that the chlorpyrifos concentration in cord blood at birth would directly contribute any to the chlorpyrifos blood concentration in children of the 1 to 2 year age. The exposure to chlorpyrifos during the 1 to 2-year period would occur mainly through food and water but also through dermal and inhalation routes because of the close proximity to the floor and the high hand-to-mouth activities of toddlers. Thus, the ongoing chlorpyrifos blood concentration between 1 to 2 years of age would be a result of the rate of direct chlorpyrifos assimilation by the child alone, and a function of operative pharmacokinetics of chlorpyrifos in that child. This exposure of the child would be largely separate from that of the mother. Overall, as the separation in time between when the cord blood sample

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was collected and when the future exposures are being predicted becomes longer, the less relevant cord blood values become to assess anyone's exposure. Clearly, children ages 1 to 2 years old are further separated in time than infants (< 1 year in age). Therefore, cord blood data cannot be used as a surrogate for assessing childhood exposure.

One utility of cord blood data for this age range, to serve as a metric of the chlorpyrifos exposure provided by the home environment in which the child lives, would be to assume that the home environment exposure over the period of interest was similar to the exposure around the time of birth. However, this is an assumption that is fraught with uncertainty. The life stage PBPK model could be used to gauge the blood concentration in children in the 1 to 2 years of age range using the chlorpyrifos dosing scenario that corresponded to the cord blood concentration. Absent the connection to the mother, this dosing scenario could likely lead to a model-predicted blood concentration quite different from the cord blood concentration. Moreover, by the time the children within the CCCEH cohort would have reached an age of two (e.g., those born in 1999 would be two in the year 2001), all residential uses of chlorpyrifos would have been cancelled and the exposure scenario would have been terminated. However, it should be emphasized that the high frequency of chlorpyrifos non-detects before 2000, and the voluntary ban of chlorpyrifos for home use subsequent to 2000, raises several fundamental question as to whether using the CCCEH cord blood data as a surrogate for assessing exposure in children ages 1 to 2 years of age is a viable risk assessment strategy.

One Panel member stated that the CCCEH study information on chlorpyrifos cord blood would be a useful and relevant source of information and a surrogate for assessing exposure to children as it would be a conservative estimate of what is likely to be higher exposure in children (age 1 to 2) given the added dietary exposures.

Question 2 - Uncertainties with Using Biomarker Data from CCCEH for the PoD (Section 7.1)

Section 7.1 describes the key uncertainties in using the cord blood biomonitoring data from CCCEH as the PoD. While biomarker data are arguably superior to conventional exposure data in that they reflect chemicals that were absorbed in the body from all routes and sources, they do not provide a direct measure of environmental exposure levels. Chlorpyrifos in blood represents a snapshot of the concentration at a particular point in time. Uncertainty also exists when establishing a quantitative relationship between chlorpyrifos concentrations in blood and adverse health outcomes. For neurodevelopmental effects investigated in these epidemiologic studies, the adverse outcome pathways, toxic moieties, and biological targets were all unknown. The key assumption is that measured biomarker levels reflect exposures during time windows that were critical for disease onset. It is also not clear whether cord blood concentrations measured at birth reflect exposure levels during the critical time window(s). However, there is a reasonable likelihood that chlorpyrifos was applied multiple times in the apartments of the women in the cohort over the course of the pregnancy (potentially once a month) increasing the potential for exposure during those unknown critical period(s). In addition, in the context of the uncertainties associated with using the CCCEH blood data in quantitative risk assessment, there is concern that the PoDs based on AChE inhibition

(Appendix 1) may not be adequately protective of human health. For example, given an external dose required to achieve 10% AChE inhibition for a female worker who was exposed dermally to chlorpyrifos 8 hours/day, 5 days/week for 3 weeks, the blood concentration of chlorpyrifos peaked at 120,000 pg/g, and was still above 100 pg/g at 32 days after the last exposure. Similarly, at a food exposure level leading to 10% AChE inhibition, chlorpyrifos concentration in blood never goes below 100 pg/g over the continuous 21-day exposure simulation and is around 7000 pg/g at the daily peaks.

Please comment on the Agency's characterization of the uncertainty associated with using the CCCEH blood data in quantitative risk assessment.

Panel Response

Overall, the Panel found significant uncertainties with using the CCCEH blood data in quantitative risk assessment. As stated by the Agency, "The key assumption is that measured biomarker levels reflect exposures during time windows that were critical to disease onset". This assumption relies on several complex unknown variables that are recognized as uncertainties by the Agency. The Panel concurs with the Agency, but has significant reservations that the steps taken during the quantitative risk assessment have sufficiently accounted for a number of key variables that would clarify these uncertainties.

First, as mentioned in the document, the time-related magnitude of exposure cannot be determined using single "spot" cord blood data. A high cord blood concentration may be the result of a low exposure sampled within hours of termination of the exposure. A low blood concentration may be the result of a much higher exposure occurring weeks prior to sampling. The toxicological impact of these two exposure scenarios could be quite different. The results being considered in the current document do not account for multiple exposure scenarios that are likely to have occurred both within and outside the home. The assumption by the Agency that the reported blood concentrations were collected during the asymptotic period when blood concentrations were fairly stable across several days would not comprise all of the many likely exposure scenarios (*e.g.*, more frequent repeated exposures or continual exposures). This, in turn, would inaccurately reflect the actual magnitude of response following these exposure scenarios.

Second, as discussed by the Agency, no particular window of exposure within the pre- or perinatal period can be identified as a key period for the effects reported in the CCCEH study, other human cohorts, or in animal studies. Without knowledge of the sensitive window(s) of exposure, it is difficult to determine the magnitude of exposure necessary to recapitulate the effects reported in the CCCEH study. The current risk assessment paradigm based on cord blood seems to treat delivery as the critical window by using cord blood concentrations obtained at that point in time to derive a PoD for neurodevelopmental outcomes. This adds an additional level of uncertainty.

There is an accumulating body of animal and *in vitro* evidence to suggest that organophosphates affect a variety of biological targets in addition to acetylcholinesterase (AChE). A few of these studies suggest that these targets may even be affected at levels that are below the threshold of AChE inhibition. However, to our knowledge, very little

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of this evidence would (so far) suggest that blood levels of chlorpyrifos in the pg/g range would have significant deleterious neurotoxicological effects in a mammalian species. Without any evidence in the animal literature or elsewhere of a mechanism of action that could explain how pg/g levels in blood could impair IQ and/or working memory, there does not appear to be biological plausibility. This is a significant uncertainty.

Several Panel members were also concerned with the lack of a clear dose-response relationship and evidence of temporality (*i.e.*, two key concepts in pharmacology and toxicology). The dichotomized "high-low" dose designation for chlorpyrifos and the related regressions (where behavioral outcome measures were plotted against highly variable blood levels of chlorpyrifos) was a source of uncertainty. The lack of "temporality" is noted above by the fact that the time-related magnitude of exposure cannot be determined using the cord blood data.

Some Panel members noted that there is considerable concern using the CCCEH cord blood data because of uncertainties raised about the analytical results. Data used for decision making should be justifiable, and, therefore, undergo rigorous review, verification and replication. Although it is acknowledged that CCCEH publications have undergone scientific peer review before publication, such review of research studies cannot be equated to studies designed under Good Laboratory Practices (GLP) or Clinical Laboratory Improvement Amendments (CLIA) standards, especially when measurements and conclusions have not been independently replicated. During the Panel meeting some questions (e.g., use of limit of detection [LOD] versus limit of quantitation [LOQ]; extraction recoveries; calibration), but not all questions, about methodology were clarified by contacting the analytical chemist who co-authored the CCCEH studies. The study (Barr *et al.*, 2002), which provided a limit of detection of 1 pg/g, was the reference cited for most of the analyses of cord blood, yet 0.5 pg/g was used as the level of detection for chlorpyrifos in later publications. Some Panel members noted that the lack of availability of raw data on individual samples, which was not provided to either the Agency or to the Panel for examination, contributed to considerable uncertainty with the analytical data. However, at least one Panel member stated that the peer-reviewed published data and results were sufficient to judge the merits of the presented analyses. Among those with concerns, replication for analyses of individual samples undergoing the same extraction procedure remains unknown and decreases confidence in data at the very low pg/g (parts per trillion) levels used to provide the arbitrary division of subjects into low and highly exposed groups based on a designated delineation of 6.17 pg/g. Confidence in such low levels would be strengthened by verification in another analytical laboratory. Although it may be deemed acceptable in certain epidemiological studies to use quantitative values when the concentration of analyte is less than detectable (*e.g.*, an analytical value of zero), such arbitrary use of analytical data for regulatory decision-making contributes more uncertainty. In addition, the use of means with large standard deviations that extend below the level of detection that are included in the analysis (for example, 3.9 ± 4.8 and 3.7 ± 5.7 pg/g in Rauh *et al.*, 2006) further decreases the value and increases uncertainties associated with the raw data that cannot and has not been independently reviewed or verified.

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Some Panel members stated that the reliance on single cord blood measurements from only one study (*i.e.*, the CCCEH study) as a primary basis for a highly impactful regulatory decision goes against standard practices of science in the fields of toxicology and pharmacology. The concept that impaired working memory and lower IQ measures observed (*i.e.,* neurological deficits) are caused primarily by a single insecticide chlorpyrifos and is predicted by the blood levels at time of delivery is not supported by the scientific weight of evidence. Peak or time weighted average concentrations during pregnancy, or a portion thereof, are more logically supportable metrics. Such metrics could, in theory, be back-calculated from the blood biomonitoring data using a validated PBPK model "if" one has data on, or can confidently make assumptions about, aspects of exposure patterns, labor and delivery, and blood collection. If such imputations cannot be made with confidence, then cord blood data should not serve as a basis for quantitative human health risk assessment.

As mentioned above, no particular window of exposure within the prenatal period or early life can be identified as a key period for the effects reported. Given this inability to identify a window, the estimated peak blood concentration or time weighted average (TWA) blood concentration within the prenatal period should be designated as the point of departure (PoD) for risk assessment, rather than using the concentration at delivery. Assumptions about exposures in the home (their timing, route, etc.), duration of labor and delivery, and blood collection will affect estimates of prenatal exposure that can (possibly) be gleaned from the blood chlorpyrifos data (and urinary 3,5,6-trichloro-2-pyridinol (TCPy) if it could be made available). On page 39 (USEPA, 2016), the Agency states "With the proposed approach, the assumption is that the CCCEH cord blood data were not collected during the period at which blood concentrations of chlorpyrifos were at their peak or rapidly changing, but instead at or near the low points on PK curves and likely during the asymptote period where blood concentrations were fairly stable across several days." The Agency has made a good case that the presence of chlorpyrifos at levels > LOD in the majority of 1998-2000 blood samples (and the occurrence in 2001-2002 at reduced frequency) reflects indoor residential chlorpyrifos use. The Agency has also made good arguments that (the vast majority of) the cord blood levels are not representative of daily peak exposures. However, the Agency's inability to confidently estimate previous exposure patterns and/or intensity hinders the use of cord blood at delivery as an anchor from which to extrapolate back to a more toxicologically meaningful internal exposure metric.

Question 3 - Pharmacokinetic (PK) Time Course: Considerations for Labor & Delivery (Section 5)

Figure 2a-b (pg. 17-18) provides an example PK profile for chlorpyrifos for current exposures to pesticide applicators. Similar figures for food, water, and residential exposures are shown throughout the issue paper (Sections 6 and 9). As shown in Figure 2a, each PK profile shows a consistent pattern of a daily, rapid increase in internal dose during the exposure period followed by a rapid decline after the exposure period ends. The rapid decline of chlorpyrifos after exposure terminates is expected given how rapidly chlorpyrifos is initially metabolized. The periods of rapid increase represent rapid uptake during activities that lead to chlorpyrifos exposures, while the periods of rapid decrease

are primarily attributed to distribution from the central compartment (circulation) into the peripheral compartments (body tissues), loss to metabolism, and binding to esterase. For chlorpyrifos, the half-life of this initial phase is estimated to be approximately four hours. Upon cessation of the exposure, the terminal half-life (approximately 120 hours) predominates resulting in an asymptotic appearance for the internal dosimetry.
As summarized in Section 5, for deriving the proposed PoDs (Section 7), the Agency is assuming the CCCEH levels do not represent values within the rapid increase/decrease phase. Instead, the Agency is assuming the reported values for cord blood and maternal blood are at the low points or within the terminal clearance period (and thus unlikely changing significantly across several days). Although part of labor is spent at home (where exposure is assumed to occur), some portion was spent in the hospital—meaning removal from the apartment caused the exposure to cease. This assumption is being made because labor and delivery typically requires multiple hours. Moreover, maternal blood samples for some mothers were taken up to two days after delivery. The Agency also notes that the results from the Agency's exposure characterization analysis of the CCCEH (Section 6) closely match those from the CCCEH study, providing further support for the Agency's characterization of the PK profile.

> ***Please comment on the Agency's characterization of the PK profile and interpretation of the CCCEH biomonitoring data. Please include in your comments the Agency's proposal to use the 10 hour and 24 hour post-peak time points on the PK profiles for assessing risk to chlorpyrifos.***

Panel Response

Overall, the Panel appreciated the Agency's transparency in their modeling efforts. Key concerns for the Panel were the claim that cord blood samples reflect an asymptotic elimination phase and uncertainty regarding chlorpyrifos exposures during and immediately after labor, and the use of cord blood chlorpyrifos concentrations at delivery as the PoD. Panel members also strongly suggested that sensitivity analyses would help identify key assumptions and parameter values.

The Panel commended the Agency's responsiveness to the recommendations of previous SAPs, as demonstrated by their willingness to embrace the challenge of applying physiologically based pharmacokinetic (PBPK) modeling techniques to human biomonitoring and epidemiological data. These are far from routine tasks, and the Agency is to be commended on their creativity, innovation, and the rigor of their efforts. Furthermore, the Agency has been transparent in the documentation of their assumptions and their efforts, and displayed openness and candor regarding the limitations of what could be achieved with the chlorpyrifos data set.

Multiple Panel members noted that PBPK modeling is, indeed, a valuable tool to interpret the biomonitoring data in circumstances where multiple routes of exposure occur, when based on best available information as inputs. Concern was raised, however, by at least two Panel members about the following four points: 1) use of cord blood at delivery in the CCCEH study as a point of departure (PoD) (rather than a simulated time-weighted average concentration during pregnancy, a peak concentration earlier in pregnancy, or even blood concentration at exit from home residence); 2) the assertion that cord blood

measurements in CCCEH can be characterized as predominantly corresponding to levels 10-24 hours post peak; 3) the lack of justification of an absence of chlorpyrifos exposure between admission and the collection of cord and maternal blood; and 4) the absence of a sensitivity analysis that would help characterize the dependence of key model outputs on particular parameters. Two Panel members commented on the level of agreement between the Agency's exposure characterization of the CCCEH and the blood measurements from the study.

Characterization of timing of the Point of Departure could be refined

The Panel did not reach consensus regarding the Agency's characterization of the cord blood data as adequately corresponding to a 10-24-hr terminal clearance phase. Concurring comments indicated that these panelists found the Agency's considerations in this regard to be careful, reasonable (but arbitrary), or adequate characterizations of the CCCEH exposure scenarios.

The dissenting panelists noted that the initial decline phase of 4 hours is less than the duration of many labors and deliveries, whereas, the Agency cited papers indicated wide variations in duration of active labor and average labors of approximately 6 hours with a weighted mean standard deviation of ~3.5 hr, placing many deliveries within the initial rapid clearance phase (~4 hr half-life) rather than within the terminal half-life phase. Specifically, the Agency's Chlorpyrifos Issue Paper (USEPA 2016; p. 19) justifies the Agency's presumption that the CCCEH cord blood levels were "at or near their low point" by stating "Neal *et al.* (2010) report than [*sic.*] among healthy, low-risk, nulliparous women at term with a spontaneous labor onset, the 'active phase' of labor lasted an average of 6.0 hours, and up to 13.4 hours at two standard deviations from the mean." However, the Chlorpyrifos Issue Paper (USEPA, 2016) fails to add that Neal also states "Perhaps the finding best indicating that the duration of normal 'active labor' varies widely is that the weighted mean of the SD was 3.5 hours." From these statistics, one can conclude[1] that approximately as many women delivered within less than 1 hour from the onset of "active labor" as delivered after 10 hours or more, as the Agency pointed out.

A short term peak will decrease by a factor of 8x in three half-lives. Thus, one can view each data point in Rauh *et al.* (2011; Figure 1A) to be the equivalent of a snapshot along a horizontal band that extends by a factor of 8x in either direction from that point depending on the individual mother's duration of labor. Much of the 6-10 hr post-exposure typical delivery time assumed by the Agency lies in a transition zone between times clearly in the initial post peak clearance phase and those in the terminal phase.

A few Panel members suggested that it does not make sense to use cord blood measurements directly; however, PBPK modeling should be used to convert the CCCEH blood data to estimate the higher pre-admission blood concentrations using the best available information on the exposure scenario. In particular, these Panel members noted

[1] An additional but somewhat peripheral conclusion is that because two standard deviations below the mean would be less than zero (not possible for this hospital admission data), the delivery data was probably not normally distributed.

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Hattis (2011) indicated that the CCCEH-study specific information on times from hospital admission to delivery and maternal blood collection, including relatively precise information about the time between hospital admission and delivery, can be expressed in hours for 133 of the mothers (Hattis, 2011; p. 50, Table 25); however, these 133 cases comprise only about one-half of the deliveries reported by Rauh *et al.* (2011). Two Panel members noted that a measure more indicative of cumulative exposure (area under the concentration vs. time curve [AUC] per day or time-weighted average concentration for a pregnant woman, or a nonpregnant woman simulated as a surrogate estimate) should be considered more relevant to risk than a single extrapolated peak concentration or a level assumed to be a 10 or 24 hr post admission concentration.

An additional panelist concurred that the measured chlorpyrifos values in cord blood are so variable as to be very poor predictors of exposure levels. This potential for variability within individual cord blood values means that a low chlorpyrifos value in Rauh *et al.* (2011) Figure 1 (*e.g.*, 1 to 2 pg/g) could have been caused by either a low recent exposure (and a low blood level as the expectant mother entered the hospital) that would not change much over the course of labor or a high recent exposure (and a high blood peak level) and a long delivery time that allowed that peak to decline to a low value. Similarly, a high value (*e.g.*, 6 to 25 pg/g in Figure 1) could have been caused by either a moderate peak and short delivery time or a high recent exposure (characterized by those in the Chlorpyrifos Issue Paper, Table 3) and a long delivery time.

<u>Assumption of no exposure after hospital admission should be justified</u>
A number of panelists questioned whether CCCEH participants were truly unexposed to chlorpyrifos for the period between hospital admission and blood collection. The Agency should provide support for this assumption, as none was identified in the current Chlorpyrifos Issue Paper (USEPA, 2016).

<u>Sensitivity analysis would inform the risk assessment</u>
Panel members who suggested use of sensitivity analysis requested both global and local sensitivity analyses that would characterize the sensitivity of PBPK model outputs (e.g., blood chlorpyrifos at key times) to model input parameters and/or exposure assumptions. Sensitivity analysis is especially important when a model is used outside of the range in which it was originally calibrated/validated. These Panel members recommend that the sensitivity analyses be extended to the maternal and fetal compartments of the Dow pregnancy model and to the Lifestage model. While it is true that a full evaluation of the modified pregnancy model is lacking – and such an evaluation would in fact present major challenges; nevertheless, it could still be used as a valuable "risk-informing" supplementary tool for calculating relevant tissue doses for fetal compartment(s) under selected scenarios.

Two Panel members had opposing viewpoints on the characterization of the realism of the Agency's exposure scenarios. One of those two Panel members noted that "the exposure characterization provided by the Agency in Section 6 closely matches the real human data that the CCCEH study has to offer." In contrast, the other Panel member stated, "If the Broadcast and Perimeter exposures occurred as assumed by the Agency,

exposed mothers would have higher blood concentrations of chlorpyrifos than those reported in Figure 1 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016). Thus, the Agency's scenarios appear to exceed the upper end of the scenarios encountered by the CCCEH study participants." A different Panel member concurred with the statement by the latter Panel member. Another Panel member noted that the words "closely match" can have various interpretations and raise unreasonable expectations; it would be more appropriate to simply say that predicted/modeled concentration ranges are consistent with the ranges of measured concentrations. (Note that there is some overlap between this portion of Charge Question 3 and Charge Question 4.)

Further individual comments not addressed in the summary

One Panel member stated:

> The Agency asks that the Panel comment "on the Agency's characterization of the PK profile and the interpretation of the CCCEH biomonitoring data." The Agency does indeed speculate on possible exposure profiles and corresponding PK profiles for CCCEH participants. However, when it comes to deriving the proposed RfD, the Agency uses PK and exposure information only to characterize the time from departing the residence until birth and maternal blood collection. Exposure patterns (timing and magnitude) of CCCEH participants probably cannot be known with sufficient certainty to derive measures of internal dose that reflect chlorpyrifos dosimetry throughout gestation. Such internal dose metrics, if they could be reliability calculated, would provide a substantially more meaningful reflection of the risk of neurodevelopmental effects than can be captured via a snapshot of blood concentration at delivery.

One Panel member stated:

> The Agency's characterization of the PK profile appropriately uses and interprets the CCCEH biomonitoring data. The Agency gives careful consideration to the context of a pregnant woman going into labor and ultimately delivering in the hospital and the time lapse between these two points. In addition, the Agency has also considered the data collected by the CCCEH cohort which provides an estimate of the time lapse between leaving the apartment, arriving at the hospital (with a reasonable presumption that any additional high level exposure ceases) and maternal blood collection (within two days) together with what is known about the terminal half-life of chlorpyrifos for an appropriate and reasonable characterization of the PK profile. It is also worthy of note that the exposure characterization provided by the Agency in Section 6 closely matches the real human data that the CCCEH study has to offer.

One Panel member stated:

(1) Further analysis of the information available in the CCCEH database could clarify various issues associated with interpreting PK data: For example, on p. 48 of the comments submitted by Dr. D. Hattis (Hattis, 2011) it is stated that "there was a generally larger average lag time (about 49 hours) between the babies' birth and the mothers' blood sampling than between hospital admission and the birth times. Overall, therefore, the total average time between hospital admission and maternal

blood sampling comes to 62.7 hours. Depending on the rate of elimination of chlorpyrifos from the mothers' blood in the interval, this lag could provide an explanation for the otherwise unusual finding that the mothers' blood levels of chlorpyrifos were not greater than the levels found in their infants' cord blood." Such information, in conjunction with PBPK model simulations, can help increase confidence in the outcomes of the analysis presented in the Agency's Chlorpyrifos Issue Paper.

(2) The PBPK model used for characterizing the CPFO PK profile is a member of the "family" of CPFO PBPK models developed over the years by Dow Agro Sciences (DAS) and DAS collaborators/contractors; it represents a mature formulation that has been extensively tested, appeared in numerous peer-reviewed publications and has been iteratively refined and evolved; recent developments include a "Multi-Route, Lifestage, and Pregnancy PBPK/PD model for Chlorpyrifos and Chlorpyrifos-Oxon" (Poet, 2015). Nevertheless, it should be recognized that the current application uses the CPFO PBPK model for conditions (e.g. ranges of very low concentrations, focus on the parent compound rather than its TCPy metabolite as the biomarker, etc.) and potential caveats/limitations associated with these issues should be identified and discussed. A systematic "global" sensitivity analysis of the PBPK model for the scenarios considered (e.g. using a standard "EPA sanctioned" method such as Morris' one-at-a-time [OAT]) would provide valuable information on the factors that affect model outcomes the most (providing substantial insight to estimates provided for two post-peak time points) and should complement the PK characterization.

(3) The Agency should consider development and maintenance of an open "community" life stage PBPK/PD modeling framework, implemented on a non-proprietary (or at least modern standard compliant) software platform, that would allow collaborative systematic and streamlined development and testing of modules for alternative hypotheses for both pharmacokinetic and pharmacodynamic processes, that will eventually consider spatial non-homogeneous tissues (in particular the brain). This would be valuable in incorporating new scientific knowledge and testing hypotheses involving presumed mode of actions (MOAs) and adverse outcome pathways (AOPs) without having to "re-invent" the wheel of modeling essential physiological/biochemical processes characterizing standardized population subgroups. Lessons learned from USEPA's past Exposure Related Dose Estimating Model (ERDEM) effort should be valuable here.

Question 4 - Evaluation of CCCEH Cord Blood Data & Predicted Exposures to the Cohort (Section 6)

The Agency has used the PBPK model to predict blood levels in women across several exposure scenarios for comparison with the cord blood levels reported by the CCCEH (Section 6). Food exposure is expected to have occurred (Section 6.2), whereas drinking water exposure was unlikely (Section 6.1). Given the lack of specific CCCEH exposure information, the Agency has developed six possible residential exposure scenarios representing a broad range of residential post-application exposures to chlorpyrifos products available prior to the voluntary cancellation of indoor products in 2000 (Section

6.3). Two exposure scenarios were conducted using EPA standard residential exposure assessment approaches; these two scenarios represent the high end exposure potential. To estimate lower exposures, four additional PBPK model simulations were conducted with use of reported values from the CCCEH investigators. These six possible residential exposure scenarios were input into the PBPK model to predict a range of potential exposures for comparison to the predicted internal dosimetry levels reported by the CCCEH investigators. Based on the results of these simulations, the Agency has concluded that: 1) the reported higher blood levels in the CCCEH from 1998-2000 are likely driven primarily by residential use of the broadcast and perimeter chlorpyrifos products registered for use at that time; and 2) these results further support the reasonableness of the magnitude and distribution of data reported by CCCEH.

> ***Please comment on the Agency's conclusions that these scenarios adequately capture the range of exposure. Please also comment on the Agency's simulations from residential and food exposures and the degree to which the estimates of internal blood levels do or do not match the CCCEH cohort results before and after the cancellation of indoor products in 2000.***

Panel Response

Overall, the Panel found that the general scenarios provided for PBPK modeling are reasonable (drinking water, food, residential).

The dismissal of drinking water as a source of chlorpyrifos during the CCCEH study seems appropriate based on the ambient monitoring data; however, those water data have limitations related to sample timing and frequency, as noted in the Agency's Chlorpyrifos Issue Paper (USEPA, 2016). It is unclear the significance of the CPFO expected, even if as stated, chlorination and mixing from other sources would dilute levels to well below limits of detection.

With regard to the simulation of chlorpyrifos exposure from food, it is promising to see that the data in Table 2 support the Agency's assertion that food exposure is likely to be low, and that these data are in general agreement with the data from the CCCEH study after the year 2000. The PBPK data for food seem to generate realistic data since they are similar to the lower levels reported in the CCCEH data, however, the peak blood level is 7.14 pg/g, which is even a little higher than the cutoff tertile reported by CCCEH (6.17 pg/g in the highest tertile in the detectable range, n=44, Rauh *et al.*, 2006). Thus, the contribution of food is probably relatively small but not completely absent.

With regard to the residential chlorpyrifos exposure scenarios, they generally capture the kinds of exposures one would expect in the residential setting. The uncertainty of the time and frequency of residential exposures in relation to the timing of cord (and even maternal) blood collection is unavoidable, and cannot be guided by the CCCEH study because those data were not available. But using monthly applications is a reasonable estimation since this was reported to be the case in the literature, and is a recommended frequency of application. It is also possible that chlorpyrifos was applied more or less frequently and it would be interesting to see exactly how more or less frequent applications would affect the data generated by the models. Focusing on post-application

exposure is appropriate. It should be noted that exposure will vary depending on whether the application is in low- or high-traffic areas.

Another area of incongruence was found in the Broadcast and Perimeter exposures. If they occurred as assumed by the Agency (maximum application rates, only chlorpyrifos used, individuals wear shorts, short-sleeved shirts, socks, and shoes, etc.), exposed mothers would have higher blood concentrations of chlorpyrifos than those reported in Figure 1.

The data presented by the Agency with respect to water and food source exposure are convincing to support the assertion that it is unlikely that these sources contribute significantly to the observed high level of measured chlorpyrifos exposure in the CCCEH cohort. Thus, it is reasonable to conclude that residential exposures are driving the observed measured levels and further, that the results of the simulations (which nicely reflect a range of residential exposure scenarios) support the conclusion that the CCCEH reported values are plausible and similar to the simulations.

Overall, the Panel found several serious sources of uncertainty in the estimates of internal blood levels and their relationship to CCCEH cohort results.

The Agency has chosen to use the "24 Hours After the Last Peak on Day-30" as the comparator for the CCCEH data. There were other, higher concentrations predicted from the models, and the Panel questions whether they should be addressed even if they are far higher than the values in the CCCEH study observed, since those higher levels may have been missed due to the timing of the blood samples in that study. This raises the question of area under the curve as a better measurement for risk assessment.

More specifically, based on the PBPK simulations that weren't available to prior SAPs, the Panel seemed to agree with the part of the assumption (USEPA, 2016; p. 20) that chlorpyrifos in maternal blood in the CCCEH samples collected 1 to 2 days after delivery is "at the low points or within the terminal clearance period (and thus unlikely changing significantly across several days)." However, other PBPK predictions led some on the Panel to conclude that most of the cord blood samples were collected during the rapid decline phase and thus are not in a similar steady-state. As a result, the measured chlorpyrifos values in cord blood are so variable as to be very poor predictors of exposure levels.

The Agency's Chlorpyrifos Issue Paper (USEPA, 2016; p. 19) justifies their presumption that the CCCEH cord blood levels were "at or near their low point" by stating "Neal *et al.* (2010) report than [*sic.*] among healthy, low-risk, nulliparous women at term with a spontaneous labor onset, the 'active phase' of labor lasted an average of 6.0 hours, and up to 13.4 hours at two standard deviations from the mean." However, the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) fails to add that Neal *et al.* (2010) also states "Perhaps the finding best indicating that the duration of normal 'active labor' varies widely is that the weighted mean of the SD was 3.5 hours." From these statistics, one can conclude that approximately as many women delivered within less than 1 hour from the

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onset of "active labor" as delivered after 10 hours or more as the Agency pointed out. Furthermore, the PBPK mathematics of half-lives and the duration of maternal labor indicate that most (perhaps as many as 84% corresponding to one standard deviation above the mean) of cord blood samples were collected during the period of rapid decline.

Neal *et al.* (2010) also states "In contemporary practice, most providers aim to admit women to the labor unit when cervical dilation is expected to become more rapid (i.e., at the onset of the active phase of labor)." Thus, it is further assumed within this context that after an expectant mother left their residence, they were no longer being exposed to chlorpyrifos from residential residues, and thus their blood chlorpyrifos levels would begin to decrease rapidly.

All of the PBPK model simulations for adults in the Agency's Chlorpyrifos Issue Paper (USEPA 2016; Figures 2, 5-8, 12-13, 16-17) show rapid phase initial half-lives of approximately 4 hours. A half-life means that one-half of the original level is still present. As the rapid elimination continues, 25% of the peak chlorpyrifos would still be present after two half-lives or about 8 hours, and 12% would be present after 12 hours. A short term peak will decrease by a factor of 8x in three half-lives. Thus, one can view each data point in Figure 1A to be the equivalent of a snapshot along a horizontal band that extends by a factor of 8x in either direction from that point depending on the duration of child labor.

This potential for variability within individual cord blood values means that a low chlorpyrifos value in Rauh *et al.* (2011) Figure 1 (*e.g.*, 1 to 2 pg/g) could have been caused by either a low recent exposure (and a low blood level as the expectant mother entered the hospital) that would not change much over the course of labor or a high recent exposure (and a high blood peak level) and a long delivery time that allowed that peak to decline to a low value. Similarly, a high value (*e.g.*, 6 to 25 pg/g in Figure 1) could have been caused by either a moderate peak and short delivery time or a high recent exposure (characterized by those in the Agency's Chlorpyrifos Issue Paper, Table 3) and a long delivery time. In fact, the full reported distribution (highs, lows, range, and mean) of the values of chlorpyrifos in cord blood reported in Rauh *et al.* (2011) could have been produced from two artificial populations: one population that delivered before the voluntary residential cancellation who all could have started "active labor" with the same relatively high cord blood chlorpyrifos values comprising short-term peaks which subsequently had the appropriate distribution of duration of labor (or time outside the home) for those peaks to have declined down to the levels actually measured; and one population that delivered after the cancellation who all started with the same relatively low cord blood with no or very low peaks but with a potentially similar distribution of duration of labor.

It is notable that relatively few of the CCCEH cord blood values in Rauh *et al.* (2011) were in the range of the peaks predicted by the PBPK model from residential exposure scenarios as summarized in Table 3 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016; p. 32). No cord blood values were reported by Rauh *et al.* that were anywhere near the "highest peak blood concentrations" of over 1000 pg/g predicted by PBPK after

residential applications. Although Rauh *et al.* (2011) indicates that cord blood values ranged up to 63 pg/g, no cord blood values above 25 pg/g were shown in their Figure 1.

One obvious explanation is the opportunity for such peaks to have decreased by a factor of 8x during in-hospital labor and thus not be visible when the cord blood sample was collected. It is easy to predict by multiplying 8 times any of the measured levels in the top tertile for Figure 1, that many such peaks could have existed at the time the expectant mother entered the hospital.

A less obvious explanation exists in the way that the cord blood results were handled. Whyatt reported in a separate correspondence (Whyatt, 2015) that four samples were above the 25 pg/g concentration at which the cord blood levels in Figure 1 by Rauh *et al.* (2011) were truncated. One of these truncated cord blood values was near the 60 pg/g peak corresponding to two of the "lowest peak blood concentrations" in Table 3, and the other three were somewhere (not specified) between 25 and 60 pg/g. Their rationale for omitting those values in both the presentation and the analysis is discussed in response to Charge Question 5.b.

As a secondary point, it turns out that some CCCEH data does exist regarding the time from hospital admission to delivery. According to Hattis (2011), CCCEH has relatively precise information about the time between hospital admission and delivery that can be expressed in hours for 133 of the mothers. The data in Table 25 (Hattis, 2011) is highly skewed toward longer delivery times than would be expected from Neal *et al.* (2010). Hattis (2011) broke the reported durations down into the difference between the calendar date of delivery and date of admission (apparently in an attempt to impute values for those births that did not have hourly data). On the one hand, the average for 59% of deliveries (the first row in his Table 25) was 5.5 hours and the average for 92% of deliveries (the first two rows in his Table 25) was 9 hours, meaning that many of the CCCEH cohort's cord blood samples were collected while their cord blood was still in its rapid decline phase. On the other hand, the duration of the next longest eight deliveries was 42 hours, and the longest two deliveries were 127 and 282 hours; the latter two are one and two 5 day half-lives into the terminal clearance phase, respectively. Unfortunately, the n = 133 of deliveries with precise information is only about half of the n = 265 cohort reported by Rauh *et al.* (2011). Hattis (2011) has started to use this time data to explore the effect described above; however further exploration may or may not help answer the questions at hand.

Another perspective of these exposure scenarios and simulations notes that based on Table 3 of the Chlorpyrifos Issue Paper (USEPA, 2016), chlorpyrifos concentrations in blood of exposed mothers would never be less than 10.7 pg/g. With a decline of ~5-10 fold during the time to delivery, typical maternal chlorpyrifos concentrations in the teens at delivery would be expected, whereas in 1998-99 (Figure 1), only about 10% of mothers were around that range, and even fewer in 2000. Per Appendix 2 of the Chlorpyrifos Issue Paper (USEPA, 2016; p. 102), 85% of respondents reported use of pest control measures, so only ~15% would lack residential exposure. The 15% value is consistent with the frequency of non-detects is 2000 [between 10 and 25%], but not for

1998-99 [0.5%]). Thus, the Agency's simulation results appear to exceed the upper end of the scenarios encountered by the CCCEH study participants. This assessment is based on the assumption that the measured values predominantly reflect the 10-24 hr post peak values.

Regarding the data quality, the use of CCCEH data when raw analytical data has not been made available, and that has not been reproduced or verified increases uncertainty.

Furthermore, uncertainty exists because levels of residential exposure are expecting a peak concentration of 60 pg/g in blood (USEPA, 2016; pp. 30-31) without verification or consideration that such blood levels would be likely occur after elimination of household use. Residential use of chlorpyrifos was banned in 2000; the values for chlorpyrifos in cord and maternal blood provided in the 2003-04 data summary of Figure 1 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016; p. 14) suggests that no samples collected after the ban tested positive for chlorpyrifos (all samples <LOD reported as 0.25 ppt). However, to make the PBPK model more conservative, it may be prudent to assume a chlorpyrifos concentration in blood comparable to that in a blood sample collected and measured before the ban on residential use, even if this contributes to uncertainty.

Question 5 - Options for Deriving a PoD for Neurodevelopmental Outcomes Based on the CCCEH Biomonitoring Data (Section 7.2)

As summarized in Section 7.2, the Agency has proposed a PoD for the observed neurodevelopmental effects and offered alternative options based on internal blood concentrations of chlorpyrifos from the results of the Columbia University study.

> **Question 5.a. Approach to Using the Cord Blood** - The Agency could consider continuing to use the AChE PoDs and apply additional factors over and above the Food Quality Protection Act (FQPA) 10X Safety Factor to reflect the level of uncertainty of protecting for neurodevelopmental outcomes when using AChE for the PoD. However, the Agency would still need to quantify the difference between effects from AChE inhibition and from neurodevelopmental outcomes—and the analysis to evaluate the appropriate additional factor(s) would again require the Agency to make quantitative use of the CCCEH cord blood data with the same uncertainties described above. The Agency has elected to propose to use the cord blood directly as the PoD as the simpler, more understandable approach. ***Please comment on the Agency proposal to use cord blood directly as the PoD.***

<u>Panel Response</u>

Some Panel members indicated that the CCCEH study is a well-designed longitudinal birth cohort research investigation that provides some of the strongest epidemiological data linking prenatal exposures to chlorpyrifos to developmental impairments later in childhood and that the longitudinal design and the measurement of biomarkers specific to chlorpyrifos at birth are a major strength of this study. However, other Panel members noted that the CCCEH study, while suggesting a link between prenatal chlorpyrifos exposure and developmental impairments, is plagued by issues that diminish the enthusiasm for this study and create a host of uncertainties. The Panel agrees that both

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epidemiology and toxicology studies suggest there is evidence for adverse health outcomes associated with chlorpyrifos exposures below levels that result in 10% RBC AChE inhibition (i.e., toxicity at lower doses). The Panel also agrees with the Agency that applying additional safety factors to the AChE PoDs to account for a possible noncholinergic MOA would be problematic because of challenges in justifying any particular value for such an adjustment.

The Agency has elected to propose to use the cord blood directly as the PoD as the simpler, more understandable approach. As data accuracy and reproducibility have emerged as major concerns across all fields of science, the Agency is asking the Panel to judge the weight of evidence based on the results from a single longitudinal study to make a decision with immense ramifications on the use of cord blood measures of chlorpyrifos as a PoD for risk assessment. One member of the Panel agrees with the Agency's simpler approach of using the CCCEH study cord blood data directly for the PoD given the concern that the PoDs based on AChE inhibition (without many additional safety factors) is not adequately protective of human health. It is a more direct approach, whilst still considering appropriate uncertainties, to use the epidemiologic data and output from the statistical analyses provided. However, as indicated in the response to Charge Question 2, the majority of the Panel considers the Agency's direct use of cord blood as inappropriate.

There is an inability to either know, or confidently make assumptions about, aspects of exposure patterns, labor and delivery, and blood collection. Chlorpyrifos measured in cord blood has been presented in the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) to represent a period of biomarker stability, where chlorpyrifos is not at peak levels or in a period of rapid decline. However, the use of the PBPK model has illuminated uncertainties for extrapolating the chlorpyrifos exposure concentration using the cord blood measures. This is in part due to uncertainty in the time between peak exposures and cord blood collection. The PBPK model results also demonstrate that the small number of relatively high cord blood levels found in the CCCEH cohort (e.g., those above 6.17 pg/g) could all have been the result of short labor and delivery times. However, no cord blood values measured within the CCCEH study even approached the 400 to 7000 pg/g magnitude of the "highest peak blood concentrations" predicted by the PBPK model to have resulted from residential broadcast applications of chlorpyrifos as shown in Table 3 in the Issue Paper (USEPA, 2016).

Some Panel members stated that transparency is an issue. The Agency uses 63 pg/g obtained from Rauh *et al.* (2011) in the Chlorpyrifos Issue Paper (USEPA, 2016) as the peak value. However, all data above 25 pg/are truncated in Figure 1A in Rauh *et al.* (2011). Some Panel members found it quite troubling that none of the four highest measured cord blood values were kept in the working memory analysis reported in Rauh *et al.* (2011), as described in response to Charge Question 5b. Neurobehavioral data were available for 3 of these 4 cord blood values. At least one Panel member, however, pointed out that the CCCEH study investigators made appropriate considerations for possible outliers and influential data points in analyses.

The uncertainty in the timing of the biomarker measurements related to developmental susceptibility (e.g., cord measures of chlorpyrifos at the time of birth may be associated with neurodevelopmental health outcomes, but may not be causal). Exposures during other periods of fetal development that might be more causally related to measured health outcomes were not measured, and there is the inability to determine the true magnitude of the exposure. In addition, there is a lack of dose dependence for the adverse biological outcome (IQ, working memory). These are key issues in the fields of toxicology and pharmacology.

There is a lack of biological plausibility or animal evidence for how picomolar (pM; 10^{-12} M) cord blood levels of >6.17 pg/g chlorpyrifos (>17.6 pM based on the CCCEH analytical results) can alter working memory and produce neurodevelopmental impairment. The mechanisms for how such potent effects can be produced at these concentrations *in vivo* are not known and have not been previously described. By comparison, the most potent selective anti-AChE drugs in current clinical use to treat deficits in working memory are known to directly engage brain AChE with inhibitory constants (IC_{50}'s) in the range of 20,000 pM (physostigmine) to 600,000 pM (tacrine). In this regard, CPFO, the active metabolite of chlorpyrifos, has an IC_{50} towards AChE of ~10,000 pM. One is left to speculate on one or more causative mechanisms having potencies more than 1,000-30,000 fold lower than cholinergic drugs known to alter working memory in patients. These estimates are conservative, since they assume chlorpyrifos levels in cord blood will directly reflect CPFO levels in the developing brain, an assumption that is currently unproven given the challenges in directly measuring the active metabolite CPFO in any tissue after exposure.

Another issue with using the cord blood directly to derive a PoD is the lack of information available to the Panel regarding the values of maternal blood used by Rauh *et al.* (2011; p. 1197) as a substitute for the 12% of the cord blood samples that were not collected. Related to that is lack of information (and thus the applicability) of the actual data used to generate the formula used to convert values of maternal blood to values of cord blood. As a result of both of these problems, the numeric values of the substituted values are unknown, as is the effect of substituted values on the PoD. And finally, as discussed in response to Charge Questions 1 and 3, the concentration of chlorpyrifos in cord blood at the time of delivery is not a reliable indicator of any given individual person's exposure.

The Agency's Chlorpyrifos Issue Paper (USEPA, 2016) may not mention the fact that apparently 8% of the mother-newborn pairs were missing a cord blood sample (Whyatt *et al.,* 2003) and 12% were missing cord blood in the data used by Rauh *et al.* (2011). The following formula was published in Whyatt *et al.* (2004) "… in cases where the umbilical cord blood sample was not collected, the mother's values were used based on the following formulas derived from regression analyses:

$$(\ln)\text{newborn plasma chlorpyrifos (CPF)} = 0.03 + 0.76\ (\ln)\text{maternal plasma CPF}$$

When that formula is taken out of the log-log domain, it can be written as follows:

$$\text{Newborn CPF} = \exp^{0.03} \times \text{Maternal CPF}^{0.76} = 1.03 \times \text{Maternal CPF}^{0.76}$$

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The result of this formula is depicted in Figure 1. As can be seen both within the formula and the figure, the relationship is not linear. Moreover, the figure shows that the ratio of maternal blood to cord blood chlorpyrifos at the highest cord blood chlorpyrifos value of 63 pg/g would be 3.5x, implicitly unequal. Without access to the original data, any limit (if one exists) on the applicable range of this formula is unknown.

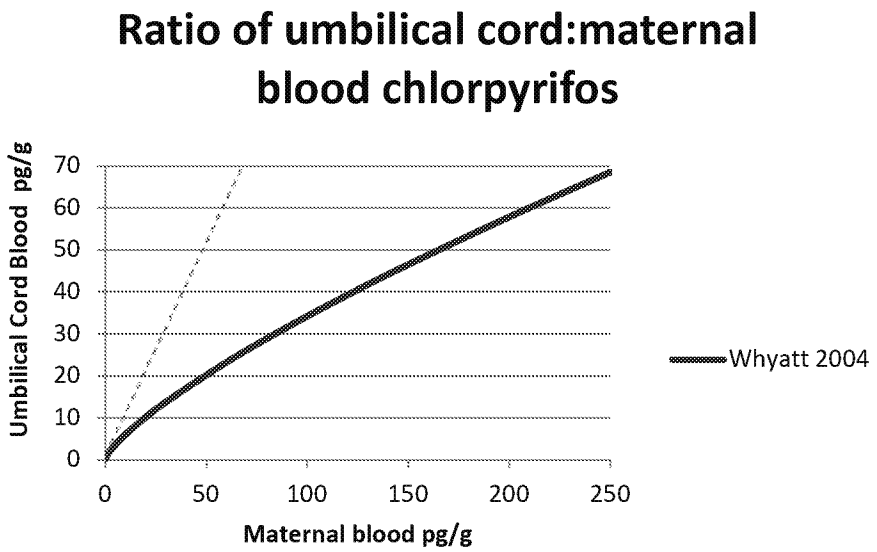

Figure 1. The red line depicts the formula presented by Whyatt *et al.* (2004) and used by Rauh *et al.* (2011) for 21 missing paired blood chlorpyrifos concentrations. The dashed line is the one-to-one ratio.

In contrast, a sense of equality was conveyed by statements within the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) on pages 13 and 17 that the "the levels of chlorpyrifos in maternal blood and umbilical cord blood levels were highly correlated," viz., r = 0.76, p<0.001, n=180 mother-child pairs in Whyatt *et al.* (2003) and $r = 0.9$, $p<0.001$, $n = 64$ in Whyatt *et al.* (2009); no explanation was given in the latter for the difference in either the "r" or "n" values. However, both of these correlations are in nonparametric Spearman rank tests, and rank correlations do not provide a quantitative basis by which such a formula can be generated. The rationale for choosing a log-log formula first by Perera *et al.* (2003) and later by Whyatt *et al.* (2004) for their missing sample formula is unknown. Although one can surmise that the formula was derived via regression, no information is provided about its confidence intervals or statistical significance. This results in an unjustified formula, based on unknown data, used to generate 21 missing cord blood data points of unknown values within Rauh *et al.* (2011), with unknown confidence intervals that are inserted in unspecified locations somewhere within the distribution of cord chlorpyrifos values proposed to be used to generate PoDs.

Unfortunately, even if the paired data were restricted to those that actually had measured cord blood (which is one way to overcome the deficiencies noted above), the evidence that much of the chlorpyrifos in cord blood was not beyond its rapid initial phase of elimination by the time of delivery (as described in response to Charge Question 3 and

summarized here) makes the cord blood data unreliable for the purposes of establishing a PoD. If the rapid phase of elimination lasted three 4-hr half-lives, then most of the cord blood samples were collected during the period of rapid decline, *viz.*, 84% using the data in Neal *et al.* (2010) and probably somewhat less using the CCCEH data shown by Hattis (2011). The PBPK model for specific routes of exposure predicts that chlorpyrifos in maternal blood (and by extension, cord blood) could vary within a range of 5x (a typical ratio between "Max Blood Levels" and "10-hour Blood Levels Post Exposure" in Table 2 for recent food exposure) to 4x to 10x (the ratio between "Lowest Peak Blood Concentration" and "10 Hours After the Last Peak" in Table 3 for recent residential exposure) within even a 10-hour range in the durations of delivery. By extending the duration of delivery to 12 hours (slightly less than two standard deviations reported by Neal *et al.* (2010), and well less than the 42 hours reported by Hattis (2011), this ratio would increase to 8x. This range of potential variability is almost as large as the entire range of cord blood values used by Rauh *et al.* (2011), and more than the range in the top tertile (>6.17 pg/g) that largely drives the slope of their regressions.

> **Question 5.b. PoD Options** – From the CCCEH publications, there are two general options that EPA has considered for deriving a PoD for extrapolating risk to chlorpyrifos: 1) Lower limit of the top tertile (>6.17 pg/g cord blood) derived from Rauh *et al* (2006) and repeated in other CCCEH publications; or 2) Benchmark Dose (BMD) estimates derived from linear regression reported in Rauh *et al* (2011) for deficits in Working Memory. Rauh *et al* (2011) reported that for each standard deviation increase in exposure (4.61 pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory. The Agency has decided to use the BMD approach.
> ***Please comment on the PoD options considered by Agency.***

Panel Response

If the Agency decides to use cord blood chlorpyrifos to determine PoD, the Agency's decision to use BMD derived from linear regression for deficits in working memory is valid. This method has been developed, reviewed, and vetted previously for methyl mercury using multiple studies. However, in this case using a single study, neither the use of the BMD nor the lower limit of the top tertile (>6.17 pg/g cord blood) as a PoD could be justified by any sound scientific evaluation. The BMD for IQ and working memory is directly linked to the cord blood values and the same concerns as noted above (in Charge 5a and in Charge 2) related to the uncertainties of relying on cord blood levels of chlorpyrifos must be considered.

One Panel member considered Option 2 (use of BMD) to be a more preferable approach as it makes fewer assumptions about the relationship between the exposure and the outcome. Option 1 is less desirable in this regard because of a somewhat arbitrary cut-off for the tertile distributions, which is appropriate for the statistical analyses, but perhaps less relevant for the Agency's purposes. Further, the use of Option 2 also allows incorporation of the variability around the single point estimate, which is another strength of this approach.

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Multiple Panel members were not satisfied with the high-low dichotomization in the analysis, and the differences in interpretation when grouped into a ND, low, mid, and high groups are also cause for concern when considering Rauh *et al.* (2006) as a possible key study. The selection of the lower limit of the top tertile seems rather arbitrary, and this basis for the exposure groupings—a carryover from an earlier preliminary study—only makes sense in the context of consistent groupings across endpoints from the same study. Describing the cutoff as the top "tertile" seems to be a misnomer, with only 50 of 254 samples in the "high group" for Rauh *et al.* (2011). In addition, the previously mentioned problems converting cord blood concentrations to something toxicologically meaningful outcome are a serious concern.

For the PoD derived based on Working Memory Index (WMI), there is no discussion whatsoever as to the biological/functional significance of any benchmark level of WMI reduction. One Panel member searched for but could not find any precedent in US EPA's Integrated Risk Information System (IRIS) for WMI reduction. Inspection of Figure 1(a) of Rauh *et al.* (2011) suggests that WMI variation at low cord blood chlorpyrifos has a standard deviation of about 10% (guessing that the mean ln WMI is 4.6, and, via counting dots, that +/- 1 SD covers the range from ~4.48 to 4.72, so WMI roughly = 100 +/- 11 (mean +/-SD)). A similar estimate was obtained from the dose-response figure of Hattis *et al.* (2014), based on an estimated SE of 1 for 145 individuals with nondetectable cord blood chlorpyrifos. By definition, the SD for an essentially unexposed population is really 15%. A 2% reduction seems to be a particularly low threshold for concluding "abnormal". If the results of Table 4 were extended up to 10 % WMI reduction, it appears the 95% LL would be ~11 pg/g (5-fold higher than the current PoD).

Paired data on WMI and cord blood chlorpyrifos (n =321) were made available to Hattis *et al.* (2014; starting at page 103). An alternative dose-response shape was derived using the data, which are depicted as groups of 145 "non-detects" and 8 groups of 22. The dose-response relationship does not look particularly "linear" in the low-dose region in Hattis *et al.* (2014) nor does it look "saturating" at higher concentrations, especially in Rauh *et al.* (2011) (see smoothed cubic splines), although data above 25 pg/g may be missing from the Rauh figure. Nonetheless, Hattis *et al.* (2014) used a Michaelis-Menten style transformation to derive dose-response parameters. Some members of the Panel would prefer to see a suite of dose-response models fit to the data (preferably the original 321 individual data points, rather than the "binned" data).

Irrespective of a cord blood chlorpyrifos level at delivery that might be selected as a cut off for a biologically meaningful risk, this value should not serve directly as the point of comparison for attained maternal blood chlorpyrifos levels in other scenarios. As noted in the response to Charge Question 5a, adjustments for fetal vs. maternal concentration and the profile of the probable blood time course should be considered.

A major issue that the Agency does not address is that the CCCEH women could have left the residence (in labor) at any point in the 720-hr, repeating cycle depicted in Agency Chlorpyrifos Issue Paper's Figure 6 (USEPA, 2016). The uncertainty in the timing of sampling relative to (1) pesticide application and (2) daily exposure maxima vs. minima

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produces too much uncertainty in the dose-response assessment. The Panel suggests that the Agency should consider the possibility that exposure in the CCCEH cannot be characterized with sufficient confidence to support quantitative dose-response assessment. As with the limit on the number of 10-fold UFs that can be applied in IRIS RfC/RfD derivations, one needs to consider a limit on extent of the conservative assumptions and uncertainties that are acceptable in applying a PBPK model to an epidemiology study.

In addition to the air sampling study (Whyatt *et al.*, 2002) and the cord blood sampling study (Whyatt *et al.*, 2003) indicating exposure of the study cohort to various pesticides, the cohort was additionally exposed to multiple contaminants including PAHs, tobacco smoke, piperonyl butoxide, and phthalates. In fact, the four chemicals listed have been demonstrated by CCCEH to affect the same parameter (*i.e.,* Bayley Scores at 36 months) (Perera *et al.*, 2003; Rauh *et al.*, 2004; Horton *et al.*, 2011; Whyatt *et al.*, 2012) that chlorpyrifos was reported to affect (Rauh *et al.*, 2006). In addition, PAHs and phthalates have been demonstrated to also affect IQ (Factor-Litvak *et al.*, 2014; Vishnevetsky *et al.*, 2015). The fact that the pregnant mothers were exposed to a complex mixture of chemicals, many of which induce deleterious effects on the same neurobehavioral parameters that chlorpyrifos is reported to affect, increases the level of uncertainty for using measurements of chlorpyrifos alone as the basis for risk assessment.

As indicated in the 2012 SAP report, the environment where the exposure occurred contained multiple organophosphate insecticides and multiple carbamate insecticides. In the CCCEH cohort, both the organophosphate insecticides diazinon and chlorpyrifos were present in 100% of the 48 hr air samples taken (Whyatt *et al.*, 2002). In fact, the air samples obtained from the pregnant mothers contained higher levels (7.5 fold) of diazinon (159ng/m^3) than chlorpyrifos (21ng/m^3) based on the mean values reported. In addition, the air samples also contained the carbamate insecticide propoxpur (85ng/m^3) in 100% of samples tested. In 2003, Whyatt *et al.* (2003) reported that both chlorpyrifos (75%) and diazinon (50%) were present in some maternal and umbilical cord blood samples. That the level of chlorpyrifos was 4 fold higher than that of diazinon is not surprising considering the greater lipophilicity of chlorpyrifos compared to diazinon. A similar pattern was again reported in Whyatt *et al.* (2004). In addition, there were 7 additional organophosphates and 4 additional carbamates detected in some of the umbilical blood samples. Because organophosphates and carbamates have the same mechanism of action (*i.e.*, inhibition of acetylcholinesterase), there is the potential for additivity to have occurred. When an individual is exposed to two (or more) chemicals that possess the same mechanism of action, the resulting toxicological outcome will be greater than if the individual was exposed to one of the chemicals alone. Thus, there was the opportunity for the pregnant mothers to be simultaneously exposed to multiple cholinesterase inhibiting chemicals. Following exposure to such a mixture, it would be biologically impossible to separate the independent effects of each chemical on a neurochemical or behavioral outcome regardless of the statistical model used.

As first mentioned in response to Charge Question 3 and again in response to Charge Question 5a, Whyatt reported in a separate correspondence (Whyatt, 2015) that only four

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samples were above the 25 pg/g concentration at which the cord blood levels in Figure 1A by Rauh *et al.* (2011) were truncated. One of these truncated cord blood values was near two of the 60 pg/g "lowest peak blood concentrations" in Table 3 that were predicted by the PBPK model, and the other three were somewhere between 25 and 60 pg/g. None of these values were included in the analysis of the correlations or the slopes reported by Rauh *et al.* (2011). Whyatt (2015) wrote that one of these four was omitted because their outcomes were not measured. The highest measured chlorpyrifos value (63 pg/g) was omitted because "this lone outlier at the extreme end of the exposure made the plot unstable and uninformative. With just two observations left in this range, the [remaining two high] data were too sparse and the splines too unstable in this region."

The Panel was assured that this treatment of data is normal in epidemiologic practice at the time (and probably conducted appropriately by the Columbia researchers). However, with the hindsight of having PBPK simulation results not available to either Whyatt, Rauh, or prior SAPs, these three data points now appear to be valid cases that could each reflect at least a portion of a recent high peak blood level while it was still in its period of rapid decline. An extended X-axis was added to a copy of Figure 1A shown below in an attempt to convey a visual sense of the leveraged role that the missing data might have played in the regression equations had that data been kept (Figure 2 below).

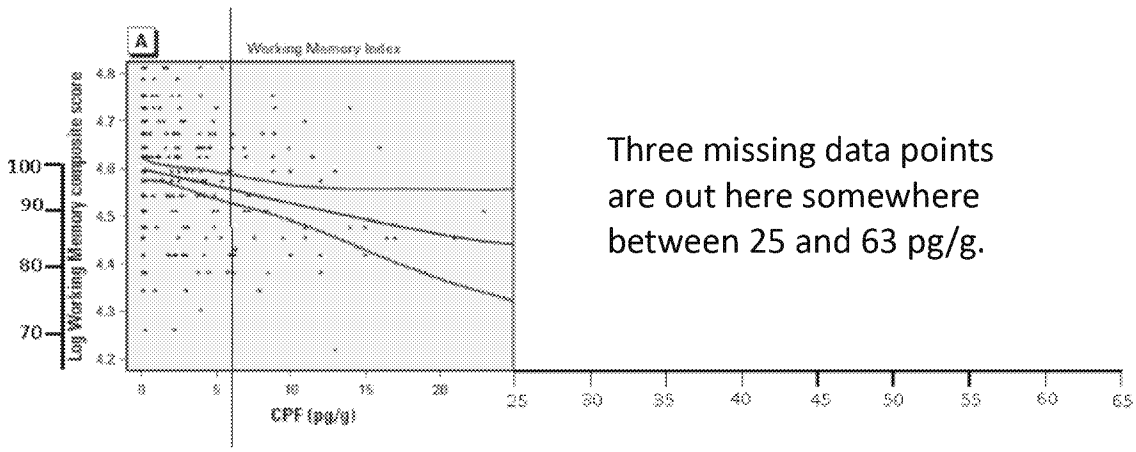

Figure 2. A copy of Figure 1A from Rauh *et al.* (2011) to which the X axis was expanded far enough to include a 63 pg/g cord blood value.

While the truncated data used by Rauh *et al.* (2011) was sufficient to demonstrate a statistically significant correlation between cord blood and WMI assuming that the null hypothesis is true (in this case, that cord blood has no effect on WMI), that does not mean that these data are also sufficient to reliably predict the slope of the dose-response relationship. In other words, the 95% confidence interval reported by Rauh *et al.* (2011) does not indicate that the slope parameter of interest has a 95% probability of being within the interval reported. This common misconception is described in such articles as Biau *et al.* (2010) and is related in part to the broader topic of reproducibility that has recently become a hot topic in scientific journals (*e.g.*, see the journal *Nature* webpage

"Challenges in irreproducible research" at http://www.nature.com/news/reproducibility-1.17552).

Thus, statistical concepts, the imprecision in cord blood, and the omission of what is now viewed as important and valid high cord blood values greatly weakens the scientific confidence in the ability of these data in Figure 1A to support a PoD (see also Charge Question 5.b.). While inclusion of the three omitted data points might in the future yield a better characterization of the dose corresponding to a chosen PoD (*e.g.*, a 2% decrease in mean WMI), the additional data will not overcome the intrinsic limitation of cord blood data imposed by the variations in delivery time discussed in Charge Question 3. Another option (previously mentioned but not recommended in response to Charge Question 3) is to access and use the information on delivery time. If the duration of time between hospital admission and delivery can be obtained, and if one assumes that cord blood and maternal blood started with equal chlorpyrifos values at the time of admission, then it may be possible to pair the cord blood value at the time of delivery within the rapid elimination phase in which a 4 hr half-life applies, with its corresponding and more temporally stable maternal blood value in which a 120 hr terminal phase half-life applies, to reconstruct the peak but unmeasured blood concentration at the time of admission. These reconstructed "zero-time" peaks should be more comparable to the peaks predicted by the PBPK model than the unadjusted cord blood values. While these zero-time peaks may still not be a better predictor of chronic exposures at much earlier times in gestation than maternal blood values, such a reconstructed value would overcome the spurious influence of the duration of labor and potentially lead to a viable PoD based on a peak concentration rather than a terminal phase concentration represented by maternal blood data.

Other options may exist to at least mitigate several other statistical problems in the way the Agency proposes to use Rauh *et al.* (2011) as dose-response data. The first of these is the uncertainty in the applicability of the average standard deviation of the slope of the chlorpyrifos dose to WMI response. The Agency proposes to rely on the statement that "On average, for each standard deviation increase in exposure, Full-Scale IQ declines by 1.4% and Working Memory declines by 2.8%." (Rauh *et al.*, 2011). The confidence interval or standard deviation of regressions are not constant and in fact are a function of the independent variable, cord blood chlorpyrifos in this case. The confidence interval is expected to become smaller for smaller values of chlorpyrifos. Thus, a single standard deviation is not appropriate for all of the percent reductions examined in the Agency's Chlorpyrifos Issue Paper Table 4 (USEPA, 2016).

> **Question 5.c. Agency's Proposal for PoD** – The Agency proposal applies the BMD approach to the Rauh et al (2011) study, and the Agency has selected a 2% change in working memory or an internal dose of 2.16 pg/g as the PoD. This Agency proposed value is quantitatively near the value reported by Rauh (2.8% reduction in working memory) and thus supported by the existing data, but is still health protective and conservative.
> ***Please comment on the analysis/calculations used to derive these estimates as described in Appendix 6 and the selection of a 2% response level.***

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Panel Response
As noted in the Response to Charge Question 5.b., a 2% response level is of questionable biological significance. Many Panel members considered a 2% change in working memory to likely be a much lower number than one standard deviation in any population of participants in behavioral studies. Other Panel members disagree. As stated in Bellinger (2004), because a population distribution moves as a whole, a small change in the mean signals predictable accompanying changes in the tails of the distribution, where individuals who meet diagnostic criteria are found. Thus, as this applies to a measure like IQ, an exposure that shifts the mean of the entire population by only a couple of IQ points will increase the prevalence of intellectual disability in that population.

It was the Panel's conclusion that the Agency provided insufficient justification for using cord blood chlorpyrifos levels and associated neurobehavioral health outcomes to derive a PoD. The cord measures of chlorpyrifos may have been associated with neurodevelopmental health outcomes but have not been demonstrated to be the sole causal factor given that other factors present could have contributed to the neurobehavioral health outcomes. Other Panel members, however, stated that the analyses and calculations used to derive the estimates are appropriate as is the extrapolation of the change in working memory.

Question 6 - Assessing Extrapolation/Uncertainty (Section 8)
In typical risk assessments, PoDs are derived directly from laboratory animal studies and inter- and intra-species extrapolation is accomplished by the use of default 10X factors. In the case of chlorpyrifos, the proposed PoDs are derived from human information obviating the need for the inter-species extrapolation. However, the Agency still needs to consider intraspecies extrapolation of the PoD from the CCCEH epidemiology data across the diverse human population (Section 8.1). Moreover, the Agency must consider the statutory requirement of the FQPA 10X Safety Factor for "potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children" (Section 8.2).

> **Question 6.a. Intra-species extrapolation** – For chlorpyrifos, the Agency proposed to use a 10X intra-species extrapolation factor. This 10X, apportioned equally between 3X for PK variability and 3X for PD variability is consistent with that used previously by the EPA IRIS program for methyl mercury (Appendix 8). ***Please comment on the Agency's scientific rationale of the proposed use of a 10X intraspecies extrapolation factor.***

Panel Response
Overall, the Panel found that the use of the 10X intra-species extrapolation factor is appropriate; however, there are other areas of uncertainty that should be considered. Uncertainty is mostly attributed to the inability of single measures of chlorpyrifos concentration in blood to provide information regarding source, frequency, duration and magnitude of exposure, and how these exposures are linked to specific outcomes measured in the CCCEH study participants.

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For intra-species extrapolation, it is clear that many factors affect differences in the epidemiology studies. PK and PD variability is almost certain to exist and will affect the PoD estimate. Using the precedent methodology set by the National Research Council (NRC) methyl mercury study is an acceptable approach, but may not be fully sufficient because of differences in the toxicology of the two chemicals, kinds of exposures, and the populations exposed. The Panel would recommend that the addition of an intra-species uncertainty factor be considered on a case-by-case basis. The methyl mercury study should not be considered a standard approach applied to all toxicant exposures, there are different types of exposures, modes of action and other factors that should be considered.

The list of sources of uncertainty listed in Table 5 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) are relevant to the CCCEH study. There are other important sources of uncertainty that should be considered:

1) The MOA(s)/AOP(s) for the neurodevelopmental outcomes related to chlorpyrifos exposures are not well defined.
2) The time between biomarker measurements in cord blood and the time when the exposures occurred is unknown (e.g., a high dose further back in time may give rise to the same cord blood levels as a smaller dose occurring less distant in time). Furthermore, the PBPK modeling results, focusing on exposures in the mothers and making assumptions about the timing and routes of exposures, indicates that the cord blood value may be an underestimate of exposure. It is unlikely that cord or maternal blood measured at birth reflect peak levels.
3) The critical neurodevelopmental window(s) are not defined (e.g., cord blood measures are being used as a proxy of previous exposures and may not be capturing the most critical exposures).
4) Any given behavioral manifestation of the toxic effects of any neurodevelopmental toxicant may depend on several types of nervous system damage and the location of this damage. Even if it was known exactly when the developing nervous system was exposed to chlorpyrifos, it is still very difficult to link that to a specific event during neurodevelopment that results in a specific behavioral deficit such as working memory or I.Q. impairment.
5) Inability to link blood concentrations with exposure doses and the lack of dose – response information for neurodevelopmental outcomes.
6) It is unknown how generalizable the CCCEH study population is for the US population in general.
7) It is uncertain how well the PBPK model accounts for differences between infants (for which the model was intended) and pregnant women (for which the model is being used—i.e. prenatal exposures).
8) The lack of definitive information on possible AChE inhibition at blood levels obtained. In animal studies doses as low as 0.36 mg/kg/d in feed were sufficient to produce maternal RBC AChE inhibition.

One Panel member stated that because of the voluntary withdrawal of residential use of chlorpyrifos in 2000, it would be highly unlikely that the CCCEH chlorpyrifos exposure scenario being used in the Agency Chlorpyrifos Issue Paper (USEPA, 2016) to derive a

PoD would ever occur again unless someone did something illegal. Several Panel members stated that the withdrawal from residential use actually functions as an additional safety factor for chlorpyrifos. Also, the Panel questioned how the Agency could regulate on levels that cannot be measured (*e.g.*, use of a PoD that provides a 10X dose that is below the LoD).

> **Question 6.b. Pre- vs. Post-natal Exposure** – Numerous epidemiological investigations have observed a link between prenatal exposure to chlorpyrifos or OPs and adverse effects on neurodevelopment through age seven years, with additional more limited evidence up through approximately age eleven years. By contrast, epidemiological evidence is more limited for associations between postnatal exposure to chlorpyrifos or other OPs and neurodevelopmental effects, and the CCCEH study has specifically not assessed these associations. Therefore, given that the extensive experimental laboratory animal database suggests that the postnatal period is a potential susceptible time, the lack of postnatal exposure assessment in the CCCEH and other similar cohort studies is a source of uncertainty in the epidemiology database.
> ***Please comment on the Agency's conclusion that the lack of postnatal exposure assessment in the CCCEH study is a source of uncertainty in the epidemiology database.***

Panel Response

Overall, the Panel supports the Agency's conclusion that the lack of postnatal exposure assessment in the CCCEH study is a source of uncertainty.

The brain continues to develop throughout infancy, childhood and adolescence. Furthermore, it is known that young children are not as efficient in metabolizing chlorpyrifos as adults (e.g., lower PON1 activity). Infants return home from the hospital to virtually the same conditions and exposures that were in the environment during the prenatal period. Furthermore, dietary exposure to chlorpyrifos has been demonstrated to be higher in young children. The fact that no critical developmental window has been identified in animal research is suggestive of ongoing adverse processes that might involve a 'second hit' hypothesis that involves an environmental trigger. The brain continues its rapid development postnatally. Neurogenesis, synaptogenesis, myelination, etc. continues in early life and brain development proceeds into adulthood, particularly frontal lobe development. This is important because impairments that have found to be associated with organophosphates (OPs) and chlorpyrifos are related to prefrontal cortex regulation, including executive function (e.g. working memory) and attention.

Taken together with the weight of evidence studies in previous human and animal studies, where exposure was limited to the postnatal period only, and which show negative neurodevelopment outcomes, it is likely that postnatal exposure to chlorpyrifos potentially contributes to its developmental neurotoxicity. The lack of such data in the CCCEH study is another source of uncertainty that should be considered.

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Importantly, since cord blood levels measured in the CCCEH study were associated with significant neurodevelopmental impairment in later childhood at chlorpyrifos exposure levels that likely would not produce 10% AChE inhibition, results from toxicology studies may be defining PoDs specific to AChE inhibition toxicity pathways that are different from underlying mechanisms related to neurodevelopmental toxicity. As a result, postnatal periods of development may be even more vulnerable to chlorpyrifos exposure than predicted in animal studies.

While postnatal periods of development may be highly vulnerable to chlorpyrifos exposure, the CCCEH study only provides evidence for a relationship between cord blood levels of chlorpyrifos and adverse neurodevelopmental outcomes in later childhood. Household usage of chlorpyrifos was voluntarily cancelled early in the CCCEH study, and significant decreases in maternal and cord blood levels of chlorpyrifos later in the study (*i.e.*, 2001-2004) provide strong support that the primary source of exposure within the CCCEH cohort was from residential usage of products containing chlorpyrifos. Since the primary source of exposure was removed early in the CCCEH study, exposures occurring postnatal in the CCCEH birth cohort would have been significantly reduced, and therefore blood levels caused by prenatal exposures provide little information on the effects of postnatal exposures on neurodevelopmental health outcomes.

> **Question 6.c. Impact of Sample Size on CCCEH Findings** – Associations with neurodevelopmental outcomes were consistently identified with respect to the number of abnormal reflexes in the neonatal period, the presence of mental and behavioral issues as well as gross motor delays were pronounced especially in at ages 24-36 months, and the observation of intelligence decrements at age seven years were seen across the three US childrens cohorts using different measures of prenatal chlorpyrifos exposure. However, with regards to dose-response, the modest sample size in the CCCEH study make it difficult to say that the dose-response relationship between exposure to chlorpyrifos and neurodevelopmental outcomes in the overall U.S. population has been fully characterized. The magnitude of the PoD in the general U.S. population of infants and children may be higher or lower than that estimated using the CCCEH study results, and the shape of the dose-response curve may also be different.
> ***Please comment on the Agency's conclusion that the moderate sample size of the CCCEH study is a source of uncertainty, given that the Agency is proposing to use the CCCEH study data directly for setting a PoD.***

Panel Response

The majority of Panel members assigned this questioned indicated that the moderate sample size of the CCCEH is NOT a source of uncertainty. Other members identified some uncertainties.

Sample size can certainly impact the interpretation of study findings and in general there is a preference to have more, rather than fewer, participants. A power calculation provides information about the magnitude of the effect. A Panel member generated a

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power curve based on a sample size of 265 participants. Those preliminary power analysis results suggest that the CCCEH had the ability to detect reductions in Working Memory Index as small as 0.1 per 1 pg/g increase in chlorpyrifos, which is much lower than the reductions they reported in Rauh *et al.* (2011) (range=0.35 – 0.81, which corresponds to a 0.0006 point reduction in log-transformed Working Memory score or a 2.8% decrease for each SD increase in chlorpyrifos). Thus, the sample size should not have impacted the study's ability to detect associations of chlorpyrifos on neurodevelopment, and more specifically subscales of the WISC-IV.

Additionally, the sample size may have limited the CCCEH study's ability to investigate effect modification, which may contribute to uncertainty as the study could not examine the association of chlorpyrifos on neurodevelopment in other vulnerable populations.

The question about whether the CCCEH results are applicable to the U.S. population has an unclear answer. Is the concern that there may be a constrained exposure range? This seems unlikely as the range of exposure was large in the CCCEH. Or is it a question of generalizability of the study? This consideration is unrelated to sample size but rather depends on the source population – in this case a Dominican and African American population. In general, internal validity is the primary goal for an epidemiologic study and the homogeneity of the study population would have reduced confounding, enhancing internal validity.

One Panel member commented that the modest sample size is a source of uncertainty given the heterogeneity of the U.S. population that is likely not captured in the study. This also limits statistical power and covariate analyses and confounding factors. However, two caveats: 1) the sample size requirements depend on the questions being asked, and 2) a large-scale study focused on the neurodevelopmental toxicity of chlorpyrifos has not be done, and may never be done because of difficulty and cost.

Another Panel member indicated the Agency's conclusion that the moderate sample size of the CCCEH study is a source of uncertainty is reasonable. While the CCCEH was sufficiently powered to detect an association between cord blood levels and neurodevelopmental outcomes within the CCCEH cohort, extrapolation of these finding to the US population in general should be done with caution given both the modest sample size and the design of the CCCEH study (e.g., restricted to African-American and Latino women residing in Northern Manhattan and the South Bronx). In addition, many cord blood measurements were near or below the assay LOD, which provides greater uncertainty in the lower range of the dose-response relationship. Given these uncertainties, the Agency's proposal to use a 10X intra-species extrapolation factor and a FQPA 10X safety factor for infants and children are warranted to ensure CCCEH findings are used to adequately protect all children in the US population.

Another Panel member noted that the issue involves not overall sample size, but the use of a small subset of values for various studies. For example, the use of 34% of a sample to measure blood lead levels (Rauh *et al.*, 2011), and the use of between 4-5 % of a

sample of children in relating high chlorpyrifos exposure and attention problems (Rauh *et al.*, 2006) introduce a level of uncertainty with respect to the conclusions of these studies.

Question 7 - Proposed Approach to Deriving Internal Dose Estimates: Integration of Exposure Assessment & PBPK Modeling (Section 9)

The Agency has proposed to input exposure estimates for chlorpyrifos into the PBPK model to assess internal blood concentrations from current exposure patterns. Several case examples were provided in the draft issue paper representing food exposures (Section 9.2), drinking water (Section 9.3), and worker exposure (Section 9.4). [Note: Exposure assumptions used in these examples have been previously reviewed by other SAPs.]

> ***Please comment on the implementation of the PBPK model using such exposure inputs and interpretation of respective simulated blood levels.***

Panel Response

The Agency developed exposure estimates for chlorpyrifos and used them as inputs for the PBPK model to assess internal blood concentrations from "current exposure patterns." Specific cases representing food exposures (Section 9.2), drinking water (Section 9.3), and worker exposure (Section 9.4) were presented and, as per the charge for this question, these cases/examples employed assumptions consistent with approaches reviewed by previous SAPs. Indeed, standard models and practices were used to develop exposure; however, the reliance on a limited number of deterministic scenarios, rather than a probabilistic population-based approach for the PBPK model simulations, raises serious questions regarding the usefulness and potential interpretation of the outcomes presented (simulated blood concentrations). The constraints of the scenarios used are compounded with the limitations of the PK profiles and dose metrics (peak blood concentration); also see response to Question 3. Although some aspects of the exposure scenarios are claimed to be improvements upon assumptions used in the 2014 Human Health Risk Assessment (HHRA) for chlorpyrifos, which employed a probabilistic/distributional framework, the current approach appears to be methodologically a step backwards compared to the 2014 HHRA.

Implementation of the PBPK Model

From dose rate theory, continuous or intermittent input of a particular dose rate (amount / day) into a model such as the PBPK model for chlorpyrifos will lead to a steady state for the internal blood concentration. When input is continuous, the steady-state blood concentration will be constant; when input is intermittent, the blood concentration will fluctuate, but the average will be the same as that produced by continuous input. The average steady-state blood concentration is the ratio of the input rate to the average steady-state clearance, provided that the clearance is independent of chlorpyrifos blood concentration; e.g., that metabolism is not saturated. The Km (Michaelis Constant, concentration that half saturates the metabolism enzyme) values for the two pathways of chlorpyrifos metabolism in the model are 1000 and 8400 ng/mL and saturation does not occur for concentrations below 10% of Km, so not below 100 ng/mL in this case. The simulated blood concentrations are in the pg/mL range so saturation of metabolism is not a concern. Other model parameter values such as tissue / blood partition coefficients and

blood flows to tissues are unlikely to be affected by the relatively low chlorpyrifos blood concentration, so the assumption that internal concentration would be linearly related to external dose rate is appropriate.

The time for the simulated blood concentration to reach steady-state is set by the model-predicted elimination half-life of chlorpyrifos, about 120 h. After dosing is initiated, the simulated blood and tissue concentrations of chlorpyrifos would rise exponentially to their steady-state values, but the time to reach 90% of steady-state would differ from tissue to tissue, with 3.3 half-lives, or 396 h for the most slowly equilibrating tissue (*e.g.*, fat). However, within a day or two the blood and highly perfused tissue concentrations are practically near steady-state (USEPA, 2016; Fig. 2a, p. 18). Input durations for the simulations were 21 days for food, 120 days for drinking water, and 14 days for worker – handler exposure. For all three inputs, the blood concentrations would be very close to steady state and accurately depict concentrations expected over longer exposures.

Exposure Inputs

For food, the chlorpyrifos ingestion rate was taken from food consumption data compiled from reported food consumption of more than 20,000 individuals over two nonconsecutive survey days during 2003-2008. Population subgroups were separately assessed: infants (< 1-year-old), children (1-2 years old), youths (6-12 years old) and adults (females 13-49 years old). The weights of the food consumed were multiplied by their respective pesticide residue values and added together to determine the quantity of pesticide consumed. That value was divided by body weight to give the exposure estimate in terms of milligrams per kilogram body weight per day. Pesticide residue values were based upon U.S. Department of Agriculture's Pesticide Data Program (PDP) monitoring data, which are measured residues in all crops for which chlorpyrifos is approved, with account taken of the fraction of total crop actually treated with chlorpyrifos. Basically the dietary input rate was based on the food that people eat in the United States and the residue content of that food, which seems to be a reasonable, data-driven approach to establishing chlorpyrifos intake rate in food. CPFO is not present in food above the limit of detection.

For drinking water, the daily consumption volume for the adult female was 1.7 liters divided into 4 times per day, and for the formula-fed infant it was 0.69 liters divided into 6 times per day. Chlorpyrifos concentration in the drinking water came from two sources: 1) a 30-year simulated concentration-time profile that would result from yearly application of 1 lb chlorpyrifos / acre to an onion field and taking account of water from precipitation and irrigation that leached the chlorpyrifos into water emitted from the field; and 2) from a daily measurement of chlorpyrifos over a year in Orestimba Creek, which received run-off from fields treated with chlorpyrifos. The chlorpyrifos water concentration-time profiles showed day-to-day fluctuations, as would be expected to occur in municipal water supply systems. Both the water volumes consumed and the chlorpyrifos concentrations in the water seemed to be appropriate and representative of what might occur in regions where chlorpyrifos is used in agriculture. Impacts of drinking water treatment on chlorpyrifos in raw drinking water were considered in detail. It was concluded that most treatments generally would not reduce the chlorpyrifos

concentration in finished water and that it was appropriate and conservative to assume that finished water had the same chlorpyrifos concentration as did the raw water. The scenarios for drinking water input of chlorpyrifos to both an adult female and an infant appear to be reasonable and realistic. However, one Panel member pointed out that Table 9 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) showed that chlorine treatment reduces chlorpyrifos by 85-90% and that at least 75% of all water treatment plants include chlorine treatment. This Panel member also noted that p. 62 of the Agency's Chlorpyrifos Issue Paper (USEPA, 2016) states that chlorpyrifos in the presence of chlorine converts to chlorpyrifos-oxon (CPFO) and CPFO is 1000X more toxic than the parent chlorpyrifos, questioning whether the conclusion above that the finished water would have the same chlorpyrifos concentration as the raw water was conservative.

The following point is made to clarify a perception rather than to identify a flaw in the water simulations. Several assumptions appear to have been made by the way the Orestimba Creek was described (ca. p. 59) in the Agency's Chlorpyrifos Issue Paper (USEPA, 2016). Creeks such as Orestimba Creek, that sometimes flow out of the Coast Range of California, are different from the streams that typify the eastern United States. The flow in such western creeks is small and seasonal. Because of this seasonality, the Orestimba Creek is not the source of any municipal water supply (and one Panel member stated that it is probably not any family's source either). Although this creek can occasionally flow into the San Joaquin River, that river, too, is probably not a source of drinking water below Friant Dam in eastern Fresno County.

While the Panel acknowledged the paucity of data on chlorpyrifos-oxon (CPFO) in water, the indication from *in vitro* experiments that it may be a thousand times more toxic than the parent compound suggests that even minute amounts of the CPFO could far exceed the impact of chlorpyrifos in drinking water (studies by Das and Barone (1999) and Howard *et al.* (2005) cited in Appendix 3, p. 118 of the Chlorpyrifos Issues Paper, USEPA, 2016).

Related to the issue of CPFO is the unknown use of bottled water within either the cohort or other target populations.

For worker – handlers, exposure-specific inputs included:

- an 8-hour workday
- two 5-day work weeks separated by two non-exposure days
- usage rates typical for the agricultural crop from currently registered product labeling
- both dermal and inhalation exposures
- exposure of 100% of the dermal surface area, and
- a daily shower following each day so no carry over of input from day to day.

This input scenario seems to be reasonable and representative of exposure that worker – handlers would encounter; however, the description of the methods could be improved. Most notable is that the statistical representativeness of the exposures leading to the venous blood chlorpyrifos for occupational handler exposure scenarios in Table 11 of the

Chlorpyrifos Issues Paper (USEPA, 2016) is not specified. Both the Pesticide Handlers Exposure Database (PHED) and the Agricultural Handler Exposure Task Force (AHETF) databases contain a range of unit exposure values for each exposure scenario. The Chlorpyrifos Issues Paper should specify which database was used and more importantly what percentile value was selected, *e.g.*, the 50th- or 95th percentiles. The phrase "... 100% of the dermal surface area was assumed to be exposed from conduct of work activities" (USEPA, 2016; p. 70) was clarified in discussion to mean that the dermal portion of the unit exposure values were obtained via full-body dosimeters. The description should also state that unit exposure values are measured while the handler was wearing a long sleeved shirt, long pants, and gloves in compliance with label instructions.

The inputs of chlorpyrifos to the PBPK model from drinking water, food, and worker – handler exposure appear to be appropriate and defensible. The inputs are well informed by a considerable amount of data relating to expected drinking water concentrations, and dietary contributions. Worker – handler inputs are informed by highly developed occupational exposure methodologies and exposure data sources that have been extensively peer reviewed. The assumptions made in development of the exposure scenarios have been reviewed previously by other SAPs. There were no compelling issues with regard to the suitability of the inputs.

Simulation Results

Table 1 below was assembled to help respond to this charge question. Based on this table, the peaks resulting from residential exposures (Table 3, p. 32) are seen to be 8x to 56x higher than the peaks produced by food (Table 2, p. 26) and 100x to 500x higher than peaks from water (Figures 12-13).

Of the three exposure modalities, the Worker – Handler scenario inputs produced the highest simulated chlorpyrifos venous blood concentrations: the average maximum peak concentrations for seven scenarios were 393 pg/g blood (range 194 – 954), average concentration after 24 hr was 5.99 pg/g (range 3.0 – 12.4), and the average concentration at 10-hr-after-last-peak on Day 12 was 31.9 pg/g (range 16.0 – 71). For the food scenarios venous blood concentrations were much lower than for the worker – handlers with the average peak concentration during the 120 d simulation being 0.67 pg/g blood at the 50th percentile and 7.14 pg/g at the 99.9th percentile. Peak blood concentration for the drinking water scenarios was similar at 7 pg/g for the onion crop scenario and 2 pg/g for the Orestimba Creek scenario.

The Agency proposes to use blood concentration of chlorpyrifos rather than input rate or dose as the reference region for definition of the RfD. An RfD of 0.022 pg/g internal concentration was proposed as the RfD based upon a PoD of 2.2 pg/g blood and a margin of error or uncertainty factor of 100. The definition of an RfD is as follows: "An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." Simulations of the worker – handler exposure scenarios produced blood concentrations 1000x above the RfD. Keep

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in mind that the RfD is not being modeled as a threshold effect, so the dose-response relationship should be able to be characterized for doses about the RfD. Big effects should be seen if pregnant women are involved in worker – handler activities.

Table 1. A compilation of maternal blood chlorpyrifos values predicted by the PBPK model and presented within the Chlorpyrifos Issues Paper (USEPA, 2016) as noted within the comments column.

	Description	peak [pg/g]	after 10 hours [pg/g]	after 24 hours [pg/g]	Comment
Water *	Adult	7		1-2	GA onion simulation with 7 µg/L spike in Figs. 12 & 14 (p. 64-66).
	Infant	20		5	
	Adult	7		<<	Orestimba with 2.2 µg/L spike in Figs. 13 & 15
	Infant	2		<<	
Food					
	50th percentile	0.67	0.14	0.05	Tables 2 (p. 26) & 8 (p. 55).
	95th percentile	1.7	0.36	0.12	
Home **		Lowest Peak			
	Broadcast	396	43	32	Table 3 (p. 32).
	Perimeter	63	13	11	
	60 pg/g on day 30	60	6-12	5-11	
Work		Maximum			
	seed planting & spray applicator	194-243	16-20	3-4	Table 11 (p. 74-5); values shown are ranges within the groups indicated.
	mixer/loader	329-413	27-38	5-8	
	loader applicator	954	71	12	

* Page 24 of the Chlorpyrifos Issue Paper (USEPA 2016) notes that "exposure to chlorpyrifos is not expected to have occurred via New York City drinking water."

** The "Lowest Peak" occurs on day 30. Day-1 peaks are higher in by 17-18x.

The Agency indicates that these are the "lower" exposure models. The Agency should also consider determination and characterization of time-weighted average blood concentrations for different exposure scenarios. The residential insecticide use scenario assumed for the CCCEH study involved repeated, monthly treatments, whereas the drinking water scenarios reflected rare, likely seasonal, events. Likewise, the worker exposures assume 2 weeks of 5 days/week exposures with a 30-day recovery period.

If the food exposures are realistic, then almost 90 percent of the adult female population always has blood chlorpyrifos concentrations above the RfD. Furthermore, this 90% of the population also, on a daily basis, experiences peak blood chlorpyrifos concentrations

13-fold above the RfD. At this RfD, half of the female population would experience peak blood chlorpyrifos concentrations 30-fold higher than the RfD. At these hazard indices there shouldn't be many "normal" children left.

The Agency presents multiple outputs from the scenarios (e.g., maximum venous blood, 24 hour venous blood, and venous blood 24 hours after the last peak), but does not indicate explicitly which of these measures would be considered risk-relevant.

Specific Comments

1. On p. 28 it is stated that "[T]he dose reconstruction analysis, including all methods and inputs, is described in Appendix 2 of this document. The goal of the dose reconstruction exercise was to estimate upper limit, bounding level exposures to test the hypothesis whether RBC AChE at or above the 10% inhibition level used by the Agency for typical AChE PoDs may have occurred in the cohort. For example, in the dose reconstruction analysis, exposure to the women was assumed to occur 24 hours a day without adjustments for bathing, showering, or leaving the residence for 14 consecutive days. In contrast, the goal of the present 2016 analysis is to predict typical product usage and behaviors thereby deriving more accurate and realistic estimates of exposure." The final statement in the preceding paragraph needs to be rephrased to reflect the limitations of any scenarios selected for simulation.
2. The wording of the title of Table 3 on p. 32, "Summary of PBPK Model Runs for Analysis of Validation of Columbia Study Blood Levels" is confusing; how can the model runs be used to validate measured blood levels? Beyond that, a column should be added to this table, showing blood concentrations 10 hours after the highest peak, so both time points (post highest and lowest peaks) can be compared.
3. On pp. 63 to 65 it is described how the chlorpyrifos PBPK model was used to predict the time course of chlorpyrifos concentrations in venous blood for an adult female with body weight of 72.9 kg (160.7 lbs.) following exposure to chlorpyrifos for two scenarios involving exposure from drinking water. The simulated adult female is assumed to consume drinking water four times a day, with a daily consumption volume of 1.71062 L (57.8 oz.) and the chlorpyrifos concentrations are based on 120 days of simulated concentration data for a Georgia-based case study (Figure 9, page 58) and on 120 days of measured chlorpyrifos concentrations from the Orestimba Creek in California (Figure 10, page 59). The peak chlorpyrifos blood concentration calculated through the PBPK model were approximately 6.99 and 1.97 pg/g. Table 10, on page 67, summarizes these results; however, it is confusing to see the 1.97 pg/g blood concentration value in a column (under "Exposure Scenario") labeled "Measured": though the water concentration was measured, the reported blood concentration is still modeled. What however, is of greater concern, is that in Table 10 the simulation results are presented not as corresponding to the hypothetical 72.9kg adult female but to the "Population" of "Females of Childbearing Age (13-49 years old)" Presenting a single estimate for women of "Childbearing Age" spanning the wide range of ages 13 to 49 is very puzzling and inappropriate. Exposure, dose,

pharmacokinetics, and pharmacodynamics for each person depend on individual physiology, biochemistry and behavior. Simple physiological factors such as body mass and BMI would have substantial impact on individual pharmacokinetic attributes (e.g. storage and release of chlorpyrifos from fat tissue and consequent shape of time-profiles). There is no meaningful average of the estimates presented in Table 10 for this "age group". A probabilistic/distributional (Monte Carlo) approach is needed in order to calculate meaningful statistical metrics for any (sub)population of concern.

4. On p. 68, under section 9.4 (Worker Exposure), it is stated that "[T]he Agency evaluated all potential occupational exposure scenarios [emphasis added] as part of the 2014 HHRA." And under section 9.4.1 (Methods), it is stated that "[I]n the risk assessment process, the Agency considers all potential scenarios [emphasis added] that could lead to exposures associated with a pesticide's use. As noted above, a comprehensive risk assessment was completed for chlorpyrifos but a select number of scenarios for pesticide handlers have been used to develop illustrative analyses for consideration. As such the methods described below focus on the types of selected exposures." The word "all" is definitely an overstatement and should be qualified appropriately to refer to "standard" scenarios.
5. On p. 77 it is stated that "the blood concentrations (Table 3) from the simulated perimeter, carpet scenario closely match the CCCEH cord blood and maternal data for 1998/1999 and 2000 (i.e., before the cancellation of indoor uses)." The words "closely match" can have various interpretations and raise unreasonable expectations; it would be more appropriate to say that predicted/modeled concentration ranges match (or are consistent with) the ranges of measured concentrations.
6. Various references mentioned in Appendix 2.0 "Summary of 2014 Dose Reconstruction Analysis" (pages 101 to 116) appear to be missing; in other cases they are unclear (e.g. reference to "Table 7-8" on page 115)

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