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Human Health Assessment Branch

DATE: August 15, 2017

SUBJECT: Response to Dow AgroSciences' Comments on the Toxicology Assessment Sections of the DPR Draft Chlorpyrifos Risk Characterization Document (dated December 31, 2015)

The California Department of Pesticide Regulation (DPR) received comments dated March 28, 2016 regarding the December 31, 2015 draft Risk Characterization Document (RCD) for chlorpyrifos (CPF). The following are responses to Dow AgroSciences' (DAS) comments related to toxicology assessment in the Risk Characterization Document.

II. TOXICOLOGY PROFILE

1. RCD Page 12:

"CPF was given a "High" priority status by the California Department of Pesticide Regulation (CDPR), due to concerns regarding… (2) genotoxicity and reproductive toxicity in rats…"

DAS Response: Chlorpyrifos is not considered a genotoxicant by any global regulatory authority and the USEPA, in its 2011 Preliminary Human Health Risk Assessment (USEPA, 2011), did not indicate a concern over this toxicological endpoint. The two studies cited (Mehta et al 2008; Rahman et al 2002) by CA DPR for evidence of genotoxicity involve the Comet assay, which is an indicator assay and does not measure apical genotoxicity effects such as DNA mutation or clastogenicity. Definitive studies on chlorpyrifos with a variety of apical endpoints that cover the spectrum of potential genotoxicity have been consistently negative. Further evaluation and comment on the Mehta et al (2008) and Rahman et al (2002) studies are noted below. Secondly, chlorpyrifos is not considered a reproductive toxicant by any global regulatory authority and the USEPA, in its 2011 Preliminary Human Health Risk Assessment (USEPA, 2011), did not indicate a concern for reproductive toxicity (reaffirmed in 2014). Moreover, in 2008, the California DARTIC reviewed chlorpyrifos for reproductive toxicity potential and concluded that "*neither the human nor animal tests by themselves met the Prop. 65 statute requirement that the "weight of evidence" clearly shows that chlorpyrifos is a reproductive toxicant.*" (Prop.65 Clearinghouse, 2008).

HHA Response: The statement on page 12 of the draft RCD describes why CPF was given a High Priority status in 2011 in DPR (http://www.cdpr.ca.gov/docs/dept/prec/2011/prec_letter_report_52_20110916.pdf).

At the time of the prioritization process, there were possible adverse effects noted in mutagenicity and reproductive toxicity studies. Based on these and other concerns, CPF entered the risk assessment process. Following review of the entire database, HHA did not consider CPF to be genotoxic, carcinogenic, or reproductive toxicant in this risk assessment.

2. RCD Page 13:

"The main targets of CPF toxicity after short-term oral exposure are the nervous system and developing organisms. Cholinergic syndromes from overstimulation of the muscarinic and nicotinic ACh receptors include hypersalivation, respiratory distress, miosis, muscular twitches, tremors, ataxia, diarrhea and vomiting."

> **DAS Response:** DAS notes that this characterization of the mechanism of action of chlorpyrifos refers to effects observed following excessive exposure, not those expected from typical ambient, real-world exposures. The USEPA has historically (and still today) based its acute and chronic exposure limits (aRfD, cRfD) for humans on red blood cell cholinesterase inhibition and not on the actual relevant biological target (brain cholinesterase) which would only occur at a much higher dose and which if inhibited to a sufficient degree, could be manifested thru symptomatology as described above. DAS recommends that CA DPR place context around this statement and would note that basing all human exposure limits on RBC cholinesterase inhibition will insure that ambient/environmental exposures are not associated with cholinergic symptoms as described by the CA DPR.

HHA Response: HHA agrees and revised the RCD accordingly.

3. RCD Page 14:

"With respect to RBC AChE inhibition, young animals are generally more sensitive than adults, and female animals are more sensitive than males."

> **DAS Response:** DAS contends that this statement needs to be contextualized relative to exposure/dose and that as stated, does not reflect the anticipated response/scenario associated with environmentally relevant exposures. Young animals are more sensitive to chlorpyrifos-induced AChE inhibition following acute exposures to high doses of chlorpyrifos (e.g., Pope et al., 1991; Pope and Chakraborti, 1992; Moser and Padilla, 1998; Moser et al., 1998, US EPA, 2011). However, young animals are not significantly more sensitive to cholinesterase inhibition than adults at environmentally relevant concentrations (Marty et al., 2012, Eaton et al., 2008; US EPA, 2011). The paucity of data on age-related sensitivity at low dose levels of chlorpyrifos was recognized by the US EPA Scientific Advisory Panel in 2008. Thus, the question on RBC AChE inhibition in young animals versus adults was examined in the

comparative cholinesterase study (Marty et al., 2012) and these data were later modeled to further examine potential differences in sensitivity. First, there was no significant difference in sensitivity to ChE inhibition between male and female PND 11 pups in the comparative cholinesterase assay (Marty et al., 2012), consistent with other reports in preweanling animals (e.g., Moser and Padilla, 1998; Moser et al., 1998). However, the comparative cholinesterase study confirmed previous reports, that the relative sensitivity of adults compared with pups was dose dependent with acute gavage exposures. At high dose levels well above levels that would induce RBC ChE inhibition and so well above the regulatory endpoint, PND 11 pups were more sensitive to CPF-induced ChE inhibition because 5 mg/kg CPF induced similar levels of plasma, RBC and brain ChE inhibition in the pups as 10 mg/kg in adults. However, at lower doses of CPF, the dose–response curves for adults and immature rats intersected, and significant RBC and plasma ChE inhibition occurred at lower dose levels than brain ChE inhibition in both PND 11 pups and adults. The NOEL for ChE inhibition across all tissues (0.5 mg/kg) was the same for both adults and pups. The enhanced sensitivity of pups to acute CPF exposure but only at higher dose levels was partially attributed to the lower metabolic capacity in younger animals (Timchalk et al., 2006). Moser et al. (1998) showed that preweanling rats have lower levels of both liver and plasma carboxylesterases and A-esterase activity than adults, which correlates with the gradual decrease in sensitivity as rats mature. However, in humans, available data indicate that liver carboxylesterase activity does not differ between infants and adults as activity appears to change relatively little during postnatal maturation (Pope et al., 2005). Furthermore, Smith et al. (2011) found no age-related differences in CPF metabolism *in vitro* using hepatic microsomes isolated from humans at 13 days to 75 years old, whereas age-dependent increases in CPFO esterase metabolism in human plasma (3 days to 46 years) were reported. In 2008, the US EPA Science Advisory Panel (2008) hypothesized that young animals might be less sensitive to **repeated** (emphasis DAS) CPF exposure due to decreased levels of enzymes converting CPF to CPFO and/or a more rapid increase in AChE activity in tissues of young animals, likely due to increased rates of protein synthesis (e.g., Chakraborti et al., 1993; Liu et al., 1999). The current study (Marty et al. 2012) verified that pups achieve higher blood levels of CPF for a given dose, presumably due to slower metabolism to TCP. Based on administered dose, these immature animals showed similar sensitivity to CPF-induced ChE inhibition as adults; however, based on blood levels, pups showed lower sensitivity to CPF-induced ChE inhibition. A recent physiologically based pharmacokinetic/ pharmacodynamic (PBPK/PD) model using human CYP-specific kinetic parameters and age-based differences in hepatic CYP content predicted that 1 year-olds would be less sensitive than 19-year olds to CPF-induced butyryl- and acetyl-ChE inhibition, although age-related differences in PON-1 levels and microsomal liver content are needed to refine the model (Foxenberg et al., 2011). Collectively, these data support the observation that young animals are not more sensitive to CPF exposure as a rule, and that often times and under conditions of actual environmental exposures, they are equally or less sensitive than adults. With respect to gender sensitivity, there is no clear indication that one gender is more sensitive than another to chlorpyrifos exposures. Adult females were either slightly more sensitive (e.g., Moser, 2000) or equally sensitive to adult males (Betancourt and Carr, 2004); in other studies, gender-related differences were not reported (Timchalk et al., 2006; Zheng et al., 2000). This

lack of consistent effects in one gender versus the other was recently recognized in the Federal Register, where the US EPA (2015) noted:

"*Overall, across the literature on neurodevelopmental outcomes and including most recent publications, there continue to be reports of effects on cognitive, anxiety/social behaviors, and motor activity. There are, however, inconsistencies in these effects with regards to dosing paradigms and gender-specificity."*

HHA Response: The sentence was modified in the revised RCD and the section was revised to incorporate DAS comments.

4. RCD Page 14:

"In 2011, U.S. EPA established a chronic BMDL of 0.09 mg/kg/day based on 10% RBC ChE inhibition in PND 11 male rats after 11 days of oral exposures."

DAS Response: DAS believes this statement to be in error since in the 2011 Preliminary Human Health Risk Assessment (USEPA, 2011), it is noted in Table 8 that the chronic point of departure (BMDL10 is 0.03 mg/kg/day based on inhibition of RBC cholinesterase in rat dams. The Revised Human Health Risk Assessment (2014) shows the results of BMD modeling of pup rat brain and RBC AChE inhibition following 11 days of repeated exposure and reports a BMDL10 of 0.09 mg/kg/day for male pups.

HHA Response: HHA acknowledges the error and it has been corrected in the RCD.

5. RCD Page 14:

Effects reported in workers chronically exposed to CPF included impaired memory, disorientation, speech difficulties, nausea and weakness.

DAS Response: The effects described would be consistent with very high exposures and extreme cholinesterase inhibition, but these were not found in an occupational manufacturing setting with exposures well above population levels (Albers, 2004; Berent, 2014). The Agricultural Health Study reported *better* neurobehavioral function among licensed pesticide applicators of chlorpyrifos (Starks et al., 2011). Any neurobehavioral effects that have been reported were determined to be unlikely related to occupational exposure to chlorpyrifos, other than those related to overt cholinergic effects related to acute poisoning, by an expert panel (Albers et al 1999). Later reviews have highlighted design problems pertinent to neurobehavioral effects in human studies (Colosio et al 2009) and lack of correlation between ChE and health endpoints (Rohlman et al 2011).

HHA Response: Please see I.E. "Human Illness Reports" in the draft RCD for further explanation of the sentence, including definitions of potential or probable associations of exposure and effect.

6. RCD Page 14:

"CPF causes developmental neurotoxicity in rats and mice at doses that elicit minimal or no fetal brain AChE inhibition."

DAS Response: In 2012, an entire EPA Science Advisory Panel (FIFRA SAP, 2012) meeting was convened, specifically to address the question of potential non-cholinergic toxicity related to chlorpyrifos exposure. Some of the charge questions and responses from the SAP panel appear below in an abridged version of the full meeting report; critical conclusions are highlighted in this summary:
Question 1.0: "…Please comment on the Agency's preliminary conclusion that AChE data remain the most robust source of data for deriving points of departure for chlorpyrifos…"
SAP Response: "The Panel concurs with the Agency's position that AChE data continue to be the strongest resource of data for deriving points of departure for chlorpyrifos….The Panel additionally notes that studies evaluating neurodevelopmental effects entailed experimental designs that do not permit an efficient means of determining point of departure for chlorpyrifos…Also in keeping with the 2008 SAP, this Panel expresses concern about the use of Dimethyl Sulfoxide (DMSO) as a vehicle…"
Question 2.1: "…Please comment on the Agency's preliminary conclusion that although there are multiple biologically plausible hypotheses being evaluated by research scientists, the mechanistic experimental toxicology data do not yet support a coherent set of key events in a mode of action/adverse outcome pathway."
SAP Response: "The Panel agrees with the Agency's conclusion that based on the current state of the science, no one pathway has sufficient data to be considered more credible than the others with respect to a causal link between chlorpyrifos exposure and neurodevelopmental outcome…"
Question 2.2: "…Given the doses/concentrations evaluated in the *in vitro* and *in vivo* mechanism studies, please comment on the degree to which these studies suggest that endpoints relevant to evaluating potential neurodevelopmental outcomes may or may not be more sensitive than AChE inhibition."
SAP Response: "The Panel concurs with the Agency that caution should be applied in interpreting the *in vivo* significance of the changes observed across the various *in vitro* studies…The Panel recommends continued literature review and analysis of published data with the goal of developing additional hypotheses linking *in vitro* findings to *in vivo* relevance…The Panel cautions the Agency concerning their examination of the dose response relationships. They particularly note that when evaluating these relationships, pharmacodynamics (PD) analyses should not be uncoupled from pharmacokinetic (PK) models given that PK differences can affect active site concentrations and hence, PD effects…Lastly, the Panel raises concerns about the equivalency of developmental stages between ages of rodents to human…This lack of equivalence further limits the translation to the *in vivo* situation and the ability to provide a quantitative dose-response relationship that can be compared to that for AChE inhibition."
Question 3.1: "…Please comment on the degree to which these studies (i.e., experimental toxicology data in laboratory rodents showing neurobehavioral effects) show changes in a

number of neurological domains and support the qualitative conclusion that chlorpyrifos exposure during gestation and/or early post-natal period may result in long—term adverse effects on the developing nervous system."

SAP Response: "The Panel agrees with the 2008 SAP conclusions that developmental neurobehavioral studies demonstrate adverse effects from chlorpyrifos exposure. However, the number of available neurobehavioral studies is limited leading to caution concerning this finding. Also many of these studies are statistically under-powered and prone to Type I errors and should be discounted in formulating the weight of evidence for or against neurobehavioral effects from developmental exposure to chlorpyrifos. The Panel also expressed caution with the significance of some of the experimental neurotoxicological outcomes that have not been validated. These included the tests of anxiety, depression, and social interactions. The Panel recommends these experimental outcomes be regarded as exploratory, and hypothesis-generating, as opposed to being evidence of toxicity…Despite the issues raised by the Panel about these studies, the overall evidence across these studies is persuasive in indicating that there are enduring effects on the Central Nervous System (CNS) from chlorpyrifos exposure at or above 1.0 mg/kg/day." Notably, this dose is greater than the NOEL for AChE inhibition at which chlorpyrifos is currently regulated.

Additional Notes (DAS): Further to the statement that DPR made about potential neurodevelopmental effects that occur at doses that elicit minimal or no fetal brain AChE inhibition, DAS notes the following. Fetal brain AChE (NOEL = 1 mg/kg/day in Mattsson et al., 2000) is not the most sensitive endpoint for AChE inhibition and does not drive the risk assessment for chlorpyrifos. The point of departure for repeat-dose chronic oral exposures to chlorpyrifos is based on RBC AChE in pregnant female rats (BMDL10 = 0.03 mg/kg/day), which occurs at lower dose levels than brain AChE inhibition across all life stages. Thus, regulations are already based on a dose level considerably lower than those affecting fetal brain AChE activity.

Question 3.2: EPA states: "…Many studies report effects at a dose of 1 mg/kg/day – a dose that produces some amount of brain ChE inhibition when given to the pups postnatally, but may or may not alter fetal brain ChE activity when given to the dams gestationally. One study (Braquenier et al., 2010) using lower doses, administered to the dam on GD 15-LD14, reported a NOEL of 0.2 mg/kg/day. Comparing the NOEL of 0.2 mg/kg/day to a repeated dosing AChE inhibition BMDL10 of 0.03 mg/kg/day suggests that AChE inhibition is a sensitive and protective endpoint." The Agency later concluded, "…no dose <1.0 mg/kg (chlorpyrifos) in any neurodevelopmental behavioral studies shows evidence of adverse effects (or of any effects, even including those outcome measures of indeterminate/unknown toxicological significance)".

While acknowledging investigative research on non-cholinergic targets, the SAP clearly acknowledged the limitations in the available study designs and data sets, as well as the unknown relationship of early pathway events (e.g., molecular initiating events, in vitro data) to adverse effects as well as the lack of understanding of dose-response relationships for key events in these pathways. Thus, regulation based on RBC AChE is a conservative approach to ensure protection to populations of all ages.

HHA Response: Please see the revised risk assessment for updated information from recent studies on the effects of CPF on humans and animals at doses equal to or lower than those

inhibiting RBC AChE inhibition. HHA reviews the available database and includes all pertinent studies in the Toxicology Profile section of the RCD as either weight of evidence or for determining PoDs. However, the studies using DMSO as a vehicle for gavage or subcutaneous CPF administration were not used in determining the critical PoDs.

7. RCD Page 20:

"The current PBPK-PD model lacks critical data on physiological changes during pregnancy and AChE genetic variability. Based on only a few human in vitro samples the model generates metabolism-related parameters that are meant to be applied to the general population."

DAS Response: To investigate the appropriateness of the default 10x DDEF, or Intraspecies Uncertainty Factor, for pregnant workers, the current Multi-Route PBPK/PD model was expanded to include systemic exposure and RBC effects predictions during all stages of human pregnancy in April 2015 (Poet 2015). This Pregnancy PBPK model was then used to validate the applicability of the new 4x DDEF for the chlorpyrifos POD in humans to pregnant women as well. Changes were made in physiology in the PBPK model based on the relevance to CPF and CPFoxon disposition and pharmacodynamics, and using well-established reference values for human pregnancy (Poet 2015, MRID 49635101). Model changes include:

- Addition of placenta and fetal compartments, which grow over the course of pregnancy.
- Pregnancy specific changes in the slow compartment, fat, and rapid compartments.
- Pregnancy specific changes in blood composition
    - Changes in blood composition result in increased blood volume, decreased hematocrit
    - Lipids, triglycerides, and cholesterol increase – leads to changes in partitioning
- Pregnancy specific changes in metabolism
    - CYP450 enzyme levels in liver
    - PON1 activity levels in liver and plasma

These important changes are included in the CPF model for pregnancy, built on the lifestage platform so either age-specific parameters or initial body weight-specific parameters can be used as the initial condition at the beginning of gestation. All model additions, changes, mathematical implementations, and model code are included in the Pregnancy PBPK model report, submitted to the US EPA in April 2015 (Poet 2015) and to CA DPR in August 2015. For all simulations in that report, either age was set to 30 years, or a body weight of 69 kg, consistent with US EPA, 2015 and the Exposure Factors Handbook mean body weight for females (U.S. EPA (2011a) Table 8-5). Enzyme activity incorporated into the PBPK model, across life-stages and in pregnant women, was based on *in vitro* measurements of CYP and PON1 rates in liver tissue and PON1 rates in plasma across a wide age range. Final ranges of enzyme activity used in the model were far wider than the measured values to accommodate a conservative estimate of variation in this critical model parameter across a human

population. Also, age-based increases in enzyme ontogenies were included in the PBPK model. Details of these model parameters are discussed subsequently in this response.

HHA Response: At the time DAS submitted the pregnancy PBPK-PD model to DPR, HHA was in the final stages of completion of its draft RCD, for which we adopted the US EPA (2014) non-pregnancy PBPK-PD modeled PoDs. We will evaluate the pregnancy model for use in estimating the internal chlorpyrifos dosimetry in the future.

8. RCD Page 20:

"Selection of RBC ChE inhibition as the critical toxicity endpoint was intended to protect human populations from impacts on other endpoints that were not easily measured. However, collective results from animal studies, the three major human prospective birth cohort studies and the ToxCast zebrafish assays indicate that CPF may cause neurodevelopmental and neurobehavioral effects in the absence AChE inhibition."

DAS Response: DAS disagrees with this broad, generalized statement that purports to link studies of various quality and not anchored by an identified and verified mode of action (in ZF, animals, humans), particularly at dose levels below the threshold for cholinesterase inhibition. CA DPR also fails to bring into the analysis any weight of evidence process such as the EPA's Draft Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment (Prueitt et al., 2012). For humans, the contention is that lower working memory in children at 7 - 11 years of age are associated with prenatal chlorpyrifos exposure and accompanying this assumption is that prenatal exposures in the Columbia University study (Rauh et al., 2006) were lower than that associated with AChE inhibition. DAS disagrees with the premise that neurobehavioral deficits in children were due to chlorpyrifos exposure and therefore disagrees with the contention that evidence exists in humans to support neurodevelopmental deficits below the threshold where AChEI occurs. For animals, there is no compelling or consistent evidence to support the contention that neurodevelopmental outcomes occur at exposures below where AChEI occurs. In many of these studies, cholinesterase has not, in fact, been measured. Most of the studies have employed doses (> 1 mg/kg/day) that are certainly associated with RBC cholinesterase inhibition and this is the conservative (protective against brain ChEI) endpoint upon which regulatory bodies globally base human exposure limits. In the case of the ZF studies and evidence that has been brought forward, there are design flaws and methodological confounders (use of DMSO as a carrier) that prevent this line of evidence from supporting the contention that chlorpyrifos in ZF is causally linked to neurobehavioral toxicity, particularly at exposures below where ChEI occurs. In fact, in many of the studies cited, ChEI was not measured and in some cases where it was, inhibition was reported, thus, contradicting the overarching statement about a causal link at low chlorpyrifos exposures.

HHA Response: Please refer to Tables 8, 13-15, 56-59 in the RCD. There is ample evidence from recent studies to support neurodevelopmental and behavioral effects at doses below those that inhibit RBC AChE.

9. RCD Page 21:

Main Uncertainties in the risk characterization were:
"(i) A default assumption for the 10-fold variation in the sensitivity (intra-species variability) within the human population was used. The default inter-species uncertainty factor of 10 was reduced to 1, because the toxicological PoDs for CPF were modeled from human data. However, for PBPK-PD modeled intra-species, the treatment levels producing a 10% change in RBC AChE inhibition was determined for an "average response", and a response at the 99th percentile of the distributions for sensitive individuals. This resulted in an intra-species Data Derived Extrapolation Factor (DDEF) of 4- and 5-fold for CPF and CPF-oxon, respectively. These predictions for variation in human sensitivity could not be used to reduce the default 10x intra-species uncertainty factor, because this model did not fully account for physiological, anatomical and biochemical changes during pregnancy. In addition, the metabolism-related age and ethnic-specific parameters (variability of PON1 and cytochrome CYP 450 enzymes) were based on a sample size that was too small to be representative of the entire population (30 human hepatic microsome and 20 plasma samples). Consequently, the default uncertainty factor of 10 was used to account for the sensitivity within the human population with respect to RBC AChE inhibition."

DAS Response: Changes to all major biological processes related to pregnancy were included in the latest revisions to the chlorpyrifos PBPK model (Poet, 2015), and described above. Inter-individual differences in PON1 metabolism have been proposed to be a significant driver in variation in biological response to chlorpyrifos exposures (Furlong et al., 2010; Huen et al., 2012). A series of local and global sensitivity analyses were used to determine critical model parameters that accounted for nearly all of the predicted inhibition of RBC acetylcholinesterase from oral CPF or CPF-oxon exposures. The distributions describing inter-individual variation in the values of the sensitive parameters were identified and included in the PBPK model. Biological and mechanistic aspects of the PBPK/PD model were then leveraged to investigate the impact of parameter variability using a two dimensional Monte Carlo analysis, and individual variability in physiological and biochemical parameters was compared to both magnitude in response variation and degree of RBC acetylcholinesterase inhibition. Results of sensitivity analysis of the chlorpyrifos PBPK model indicate that PON1-mediated clearance of CPF-oxon is an important determinant of variation in RBC acetylcholinesterase inhibition after exposure to either CPF or CPF-oxon (Price, 2013; Poet, in preparation). Coefficients of variation for metabolic rates for this PON1 activity, in liver and plasma, as well as hepatic CYP450 metabolism of the parent CPF were based on measured *in vitro* rates (Smith et al., 2011). Conservative estimates of the final variation in the critical model parameters (including metabolic rates) were then determined via bootstrap analyses. The results of these bootstrap analyses afforded ranges of CPF activation and CPF and CPF-oxon metabolic clearance that are significantly wider than the *in vitro* values measured by Smith et al. 2011 (Poet et al. 2017; Price and Poet 2013). As shown in Text Table 1, ratios of maximum/minimum values for hepatic and plasma PON1 activity used in the PBPK model are both 58, which is 5-10 fold wider than the empirical data of Smith (2011).

Text Table 1. Ratios of the maximum to minimum value in the raw data and bootstrap model simulations for the critical enzyme activities.

	CYP450 to TCPy	CYP450 to Oxon	Hepatic PON1*	Plasma PON1*
Range in *in vitro* data (Smith et al., 2011)	12	28	11	6
Range used in PBPK model for DDEF calculations (from 20 parametric bootstraps)	74	98	58	58

* Values for PON1 in liver and plasma were assumed to be correlated and thus have the same variation.

While genetic variation in the PON1 gene can alter the catalytic efficiency toward organophosphorus compounds (Albers et al., 2010; Costa et al., 2013), it is also important to understand that these differences in the metabolism of CPF-oxon have been recently shown to be modest or non-existent at relevant environmental contaminant levels (Coombes et al., 2014). The lack of phenotype impact on chlorpyrifos-oxonase activity is also suggested by the relatively narrow range of activity toward this substrate compared to paraoxon reported by Huen et al., (2012) (34-fold and 165-fold for CPF-oxon and paraoxon, respectively). Due to this lack of differentiation, a single log normal distribution (bounded at the lower end at one percent of the mean rate) was used to describe PON1 activity. Age-based changes in the critical metabolic parameters (CYP and PON1) are also included in the PBPK model. These changes are based on the *in vitro* study of Smith et al. (2011). These authors found a substantial age-based increase in plasma PON1 activity from infants through adulthood. This increase in plasma PON1 activity with age which was incorporated into the PBPK model (Figure 1).

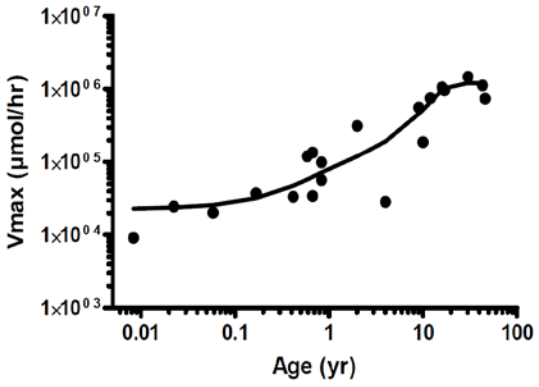

Figure 1. Total Vmax values of human enzymatic metabolism of CPF-oxon to TCPy in plasma over various ages (From Dow AgroSciences, 2011).

These authors also investigated hepatic metabolism of chlorpyrifos and the oxon metabolite across ages (Smith 2011). Little to no changes in metabolic rate for CYP conversion of chlorpyrifos to oxon, CYP conversion of chlorpyrifos to TCPy, or PON1 hydrolysis of oxon to TCPy were found across ages, based on units of activity per mg of microsomal protein.

However, when these rates are incorporated into the PBPK model, along with age-dependent changes in liver organ volume (Young 2009), the following predicted increases in the various metabolic rates are seen across ages (Figure 2).

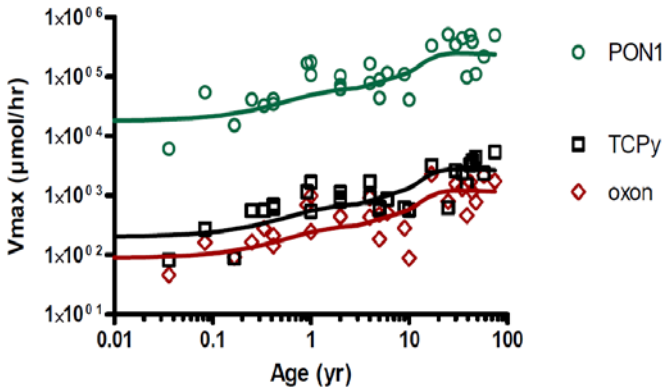

Figure 2. Hepatic metabolism of CPF to TCPy (CYP), CPF-oxon (CYP) and of CPF-oxon to TCPy (PON1) increases with age. Note: total Vmax is on a logarithmic scale (From Dow AgroSciences, 2011).

In summary, the PBPK model incorporates a broad range of variability in predicted PON1 activity (58-fold), to adequately (and conservatively when compared to available empirical data) simulate the variation in this enzymatic step of chlorpyrifos oxon hydrolysis in humans. The PBPK model also incorporates substantial metabolic rate changes with age, in both plasma PON1 activity as well as hepatic rates of CYP and PON1 metabolism.

HHA Response: We appreciate your clarification about the variability incorporated into the model and we found it useful to add this information to the RCD. It was helpful in the rationalization of the current intraspecies UF related to the PBPK-PD model (for the reasons listed in the RCD). We have updated the RCD to include the variability for the 4 critical parameters listed in your comments and in Smith et al. (2014).

10. RCD Page 22:

"Evidence from human epidemiological and animal toxicology studies showed associations between fetal and early life exposure to CPF and long-term neurodevelopmental and neurobehavioral effects. Mechanistic studies in animals using pathway-based analyses revealed that CPF irreversibly affected neurogenesis and nervous system development in fetuses as well the developing organisms."

DAS Response: There are no citations or references of support for this statement obtained from the CA DPR RCD. This notwithstanding, CA DPR's consideration of experimental toxicology studies in conjunction with reported epidemiological findings does not serve as a suitable and unifying basis for a weight of evidence determination that chlorpyrifos may be associated with claims of neurodevelopmental effects in humans (Prueitt et al., 2011; Li et al., 2012). Importantly, numerous experimental animal studies purporting to associate

chlorpyrifos with neurodevelopmental effects have been reviewed by several EPA SAPs and which have concluded that no mode-of-action (MOA) or adverse outcome pathway (AOP) can be defined or described. The epidemiological studies are inconsistent in reported findings and the raw data and study details have never been publically presented or made available for independent review. A vast majority of the experimental studies that are cited as forming the basis for a proposed linkage between chlorpyrifos and effects on neurodevelopment have methodological/design challenges which severely limit their utility in a weight-of-evidence assessment. These factors include dose, inappropriate route of exposure, and utility of a neurotoxic vehicle (i.e., DMSO), factors which have been highlighted by USEPA Scientific Advisory Panels (SAP's) as limiting the utility of reported results when considering relevance to humans. Many of the experimental studies (both *in vitro* and *in vivo*) cited as forming the basis for the alleged association between chlorpyrifos exposure and effects on neurodevelopment in children use dose levels that are tens of thousands of times higher than actual human exposures and hence there is little relevance of these studies when considering risk to humans. Several SAP reviews in addition to the USEPA RHHRA are consistent in their finding that a chlorpyrifos mode-of-action/adverse outcome pathway (MOA/AOP) leading to neurobehavioral effects cannot be established. This has led EPA to state that "*uncertainties such as the lack of an established MOA/AOP for neurodevelopmental effects and the potential exposure to multiple-AChE-inhibiting pesticides preclude definitive causal inference*." In summary, there is an absence of compelling, consistent scientific (human or animal) evidence or a proposed, tested, and validated mode of action to support the contention that chlorpyrifos is associated with neurodevelopmental effects in humans.

HHA Response: We purposely did not include references in the Executive Summary. Our points are fully supported in the Toxicology Profile, Hazard Identification, and Risk Appraisal sections.

11. RCD Page 22:

"In the zebrafish model, CPF also caused irreversible neurodevelopmental and neurobehavioral deficits many of which were unrelated to brain and RBC AChE inhibition."

DAS Response: As already stated above, the vast majority of the experimental studies that are cited as forming the basis for a proposed linkage between chlorpyrifos and effects on neurodevelopment and neurobehavior have methodological/design challenges which severely limit their utility in a weight-of-evidence assessment. These factors include dose, inappropriate route of exposure (de-chorionation), utility of the neurotoxic vehicle DMSO, lack of solvent controls, inadequate replication, and a lack of analytical confirmation of the exposure concentrations.

HHA Response: HHA disagrees that the zebrafish model is not appropriate. We find that the model is a useful source of weight-of-evidence data. Published articles have shown that DMSO is not neurotoxic in zebrafish at the vehicle concentrations used in dosing studies (Maes et al. 2012). DMSO must exceed 2% or greater to be neurotoxic, however concentrations used in

zebrafish studies are generally 0.1% or less. The models used in the RCD were standardized and have been incorporated into the ToxCast/Tox21 Program.

12. RCD Page 22:

"Based on preliminary estimates of the oral in utero PoDs for working memory decrements in children 7 yrs old, the threshold for disruption of the endocannabinoid or serotonergic systems in rats and the active concentration causing cognitive, anxiety and learning deficits in zebrafish the neurodevelopmental effects could be predicted to occur at doses 3-10 fold lower than AChE inhibition."

DAS Response: DAS disagrees with this statement that purports to link studies of various quality and not yet anchored by an identified and verified mode of action (in ZF, animals, humans) particularly at dose levels 10X below the threshold for cholinesterase inhibition. CA DPR also fails to bring into the analysis any weight of evidence process such as the EPA's Draft Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment (Prueitt et al., 2012). In humans, the contention is that lower working memory decrements in children at 7 - 11 years of age are associated with prenatal chlorpyrifos exposure and accompanying this assumption is that prenatal exposures in the Columbia University study (Rauh et al., 2006) were lower than that associated with AChE inhibition. DAS disagrees with the premise that neurobehavioral deficits in children were due to chlorpyrifos exposure and therefore disagrees with the contention that evidence exists in humans to support neurodevelopmental deficits below the threshold where AChEI occurs. For animals, there is no compelling or consistent evidence to support the contention that neurodevelopmental outcomes occur at exposures 3-10X below where AChEI occurs. In many of these studies, cholinesterase has not, in fact, been measured. Most of the studies have employed doses (> 1 mg/kg/day) that are certainly associated with RBC cholinesterase inhibition and this is the conservative (protective against brain ChEI) endpoint upon which regulatory bodies globally base human exposure limits. In the case of the ZF studies and evidence that has been brought forward, there are design flaws and methodological confounders (use of DMSO as a solvent) that prevent this line of evidence from supporting the contention that chlorpyrifos in ZF is causally linked to neurobehavioral toxicity, particularly at exposures 3-10X below where ChEI occurs. In fact, in many of the studies cited, ChEI was not measured and in some cases where it was, inhibition was reported, thus, contradicting the overarching statement about a causal link at low chlorpyrifos exposures.

HHA Response: HHA maintains the position that, while there is not a specific MOA or AOP for CPF, there is sufficient data in humans and animals to indicate that effects are occurring at doses or exposures below those which results in AChE inhibition.

13. RCD Page 27:

"Since then the SAP encouraged the U.S.EPA to evaluate current cholinergic (AChE) and noncholinergic adverse endpoints, including developmental neurotoxicity and cognitive/behavioral alterations from CPF exposure (U.S. EPA and /SAP 2012)."

DAS Response: While encouragement has been noted from the SAP for continued exploration of non-cholinergic modes-of-action, to date, no viable, tested, and validated pathways linking chlorpyrifos exposure to neurodevelopmental/neurobehavioral endpoints or outcomes have been identified. DAS agrees with the 2008 SAP that the literature that has explored and reported on neurodevelopmental or neurobehavioral findings *in vitro* and *in vivo*, is not anchored or supported by an identifiable, validated, and replicated biologically plausible mode of action at levels below the threshold for AChE inhibition. This is also a critical point to the human relevance framework and whether results in animal studies can and should be extrapolated to humans. The 2008 SAP panel concluded the following relative to this question (2008 SAP Minutes at 28):

"There was a consensus of the Panel that available data were inadequate to support a weight of evidence evaluation for non-cholinergic mode(s) of action for the behavioral alterations following gestational and early postnatal exposure to chlorpyrifos that persisted into adulthood. The Panel agreed that the available information does not allow for behavioral endpoints to be considered as a point of departure and recommended, based upon currently available data, that cholinesterase inhibition be used as the PoD". And then from the 2012 SAP (2012 SAP Minutes at 15), the Panel stated that: *"[T]he number of available neurobehavioral studies is limited leading to caution concerning this finding. Also, many of these studies are statistically under-powered and prone to Type I errors and should be discounted in formulating the weight of evidence for or against neurobehavioral effects from developmental exposure to chlorpyrifos. The Panel also expressed caution with the significance of some of the experimental neurotoxicological outcomes that have not been validated. These included tests of anxiety, depression, and social interactions. The Panel recommends these experimental outcomes be regarded as exploratory, and hypothesis-generating, as opposed to being evidence of toxicity. The lack of specificity in the direction of the neurobehavioral dose response findings is a problematic issue".*

HHA Response: HHA maintains that more recent findings and published studies indicate a growing association between CPF exposure during gestation and/or development and neurobehavioral/neurodevelopmental toxicity at doses lower than those inhibiting brain AChE.

14. RCD Page 33:

"The toxicological significance of plasma and RBC AChE inhibition is less certain because the physiological function of ChEs in blood have not been clearly established, although several possible physiological functions have been proposed."

DAS Response: DAS is not aware of any generic or specific physiological function associated with RBC cholinesterase, nor does CA DPR provide any citation to support this statement and contention.

HHA Response: The paragraph in the RCD where this original sentence is found (page 33 of the RCD) further elucidates potential associations and cites numerous references including Lockridge and Masson 2000, Brimijoin 1992, Ballard and Perry 2003, Giacobini 2003, and Li et al. 2000.

15. RCD Page 34:

"Although blood ChE (plasma BuChE and RBC AChE) inhibition is not usually detrimental, it can be used as a surrogate for brain and/or peripheral AChE inhibition when such data are lacking…"

DAS Response: DAS is not aware of any studies/data that would support the inference that inhibition of blood ChE may be detrimental or is associated with any toxicodynamic response. It is well-recognized that inhibition of blood ChE occurs well prior to inhibition of brain ChE and that it is a conservative surrogate (upstream) for brain/peripheral nervous system ChE inhibition.

HHA Response: The RCD has been revised to reflect the DAS comment.

16. RCD Page 47:

"However, studies performed with CPF, using the comet assay (Mehta et al. 2008; Rahman et al. 2002), showed DNA damage. Mehta et al. (2008) treated male Wistar rats with CPF for 1-3 days at 50 or 100 mg/kg/d or for 90 days at 1.12 or 2.24 mg/kg/d. Results showed increased DNA damage in liver and brain at all doses tested in all dosing regimens. Rahman et al. (2002) tested CPR for the ability to induce in vivo genotoxic effect in leucocytes of Swiss albino mice using the single cell gel electrophoresis assay or comet assay. The mice were gavaged with CPF (0.28 to 8.96 mg/kg) body weight and whole blood leukocytes were examined at 24, 48, 72 and 96 h. A dose-related increase in mean comet tail length indicating DNA damage was observed at 24h post-treatment (P<0.05) with CPF in comparison to control. By 96 h post-treatment the mean comet tail length reached control levels indicating repair of the damaged DNA."

DAS Response: Because these studies were not conducted according to the current OECD guideline, because of questions on the study design and other experimental parameters (e.g., vehicle), DAS questions their use and relevance for regulatory decision-making. DAS has evaluated the Rahman et al (2002) and Mehta et al (2008) studies and offers the following comments. In the Rahman et al (2002) study, the study design is not clear nor is there any information provided about the vehicle employed. The treatment schedule was not provided and hence it is not known whether this was a singular or multiple dosing regimen. DAS would also note that the Comet measurement is not compliant to an OECD guideline. OECD guideline recommends % Tail DNA, rather than Comet tail length; additionally, information about how the data were summarized for statistical analysis is not available in the paper. Because the study is not compliant to the current OECD guideline and the study design is not

thoroughly described, the significance of reported positive observations is questionable. While questions remain on the study itself, the results clearly demonstrate the reversibility (repair) of the DNA damage detected by Comet assay. "*Our investigation has shown that there was a time dependent decrease in DNA damage after treatment with both chloropyriphos [sic] and acephate. From 48 h post-treatment, a gradual decrease in mean comet tail length was found for all doses of both pesticides, indicating DNA repair*". Importantly, the Comet assay is an indicator assay and does not measure apical genotoxicity effects such as DNA mutation or clastogenicity/aneugenicity. As such, positive Comet results can be confounded by biological effects such as DNA repair or cytotoxicity and does not necessarily mean heritable mutagenicity. Relative to the Mehta et al (2008) study, DAS would note that the dosing route (i.m.) is not relevant to human exposure and that the acute doses (50 and 100 mkd) are excessively high compared to anticipated environmental human exposures which logically then brings into question human relevance. Cytotoxicity was not evaluated and the data analysis of the acute exposure was not based upon the animal number (n=3), but the data points (n=9, 3 repeats per sample), which mistakenly increases the statistical power. As noted previously, the Comet measurement is not compliant to the OECD guideline as the OECD guideline recommends % Tail DNA, rather than Damage Index. Additionally, no concurrent positive control group was included in the study; thus the damage extent relative to a positive compound cannot be evaluated. The study is not compliant with the current OECD guideline and the Comet assay is an indicator assay and does not measure apical genotoxicity effects such as DNA mutation or clastogenicity/aneugenicity. As such, positive Comet results can be confounded by biological effects such as DNA repair or cytotoxicity and does not necessarily mean heritable mutagenicity.

HHA Response: Results from genotoxicity tests based on FIFRA guidelines and non-guideline studies were mostly negative. However, CPF caused DNA damage in yeast and bacteria and in two *in vivo* comet assays. HHA has added additional discussion in the revised RCD addressing the positive genotoxicity assays including the Comet assays. Please note that HHA evaluates registrant-submitted studies according to the US EPA Health Effects Guidelines and not according to the Organisation for Economic Co-operation and Development (OECD).

17. RCD Page 49:

"There is an acceptable Health Effects Test guideline CPF developmental neurotoxicity study (DNT) submitted by the registrant as well as open literature studies. These studies are detailed in the HHA Summary of Toxicology Data (APPENDIX 1) and in the U.S.EPA risk assessment documents (U.S.EPA, 2007, 2011 and 2014). Table 14 focuses on neurobehavioral effects in pups after rat or mouse pregnant dams and their preweaning pups were treated with CPF by oral gavage, subcutaneous injection or dermally. Some citations overlap with those in Table 13 but the focus in Table 14 is on neurobehavioral effects."

DAS Response: It is important to critically assess studies published in recent years with respect to test design, dose, route of exposure, vehicle, and reported effects to determine

whether there is both biological plausibility and coherence of findings relative to putative neurodevelopmental effects associated with chlorpyrifos exposure, some of the studies which are contained in CA DPR Table 14 (2015). It is well-acknowledged that many of the laboratory animal studies that have reported findings associated with neurodevelopment, including behavioral and cognitive effects, utilize a route of exposure (subcutaneous; many contained in Table 14) that is not relevant to humans and which may result in more rapid and different delivery of chlorpyrifos to the systemic circulation, which ultimately reflects an artificial and unrealistic situation compared to the human scenario. In addition, there has been robust and consistent caution by both investigators and the USEPA Scientific Advisory Panel (2008; 2012) on the use of DMSO as a vehicle for delivery in *in vivo* studies. For example, the OECD 2007 DNT 426 guideline specifically states: "*The vehicle should not cause effects that could interfere with the interpretation of the study, neither be neurobehaviorally toxic…*" However numerous animal studies that are used as the basis for reported neurodevelopmental concerns in humans use a vehicle, subcutaneous DMSO, that possesses neurotoxic properties at the doses used (1 mL/kg). The kinetic and neurobehavioral properties of DMSO present a significant confounding variable when neurotoxicity and neurodevelopment are the key endpoints of valuation/investigation. Cavaletti et al (2000) have shown that the administration of dilute solutions of DMSO can have a significant impact on the nervous system. They note that "*The neurophysiological and pathological changes observed in our study are severe enough to merit careful consideration in the course of experimental studies involving DMSO as a solvent for drugs which are under evaluation for their potential neurotoxicity*." Other authors have shown that DMSO used as a dose vehicle can also enhance the clinical symptoms of organophosphates (Ballough et al. 2008; Carr et al. 2008). In the 2012 EPA SAP, the Panel stated that "*in keeping with the 2008 SAP, this Panel expresses concern about the use of Dimethyl Sulfoxide (DMSO) as a vehicle because of its intrinsic toxicity, its potential influence on absorption and interaction with chlorpyrifos, and the impact of this interaction on the developing organism*." While the EPA presented *in vitro* and *in vivo* study evidence in the RHHRA (EPA, 2014) that supported their proposed position that chlorpyrifos likely played a role in the neurodevelopmental outcomes in the Columbia Study, they recognize the challenge with dose levels used in these experimental studies. As stated in the RHHRA at page 158: *"In summary, in the late 2000s, a number of papers were published on the in vitro modification of various proteins by chlorpyrifos or chlorpyrifos oxon. Although interesting and provocative, these studies were usually conducted with exceedingly high concentrations (high micromolar to millimolar) of the OP compound, making the connection to a 'real world' human exposure tenuous."* The SAP (2008) recognized some of the challenges that accompany these investigative studies, particularly for how they might or might not assist with human health risk assessment. They noted that the following. The 2008 SAP Minutes at page 12 states: *"Some members questioned the experimental methods used in some of the animal studies as well as the interpretation and application of the results of neurobehavioral testing in animals for risk assessment. It was acknowledged that the study outcomes could be affected by 1) the route of administration of chlorpyrifos, 2) the developmental period of exposure, 3) the methods used to measure changes in behavioral domains, and 4) the choice of dependent variables. Panel members agreed with the Agency's expressed caution on the use of dimethyl sulfoxide (DMSO) as a vehicle because of its intrinsic toxicity and potential influence on absorption.*

In addition, uncertainty was expressed about potential interactions between DMSO and low doses of chlorpyrifos and the effect of this interaction on the developing animal." It is notable that many of the studies cited in the CA DPR Table 14 used DMSO as a vehicle. DAS agrees with the 2008 SAP that the literature that has explored and reported on neurodevelopmental/neurobehavioral findings *in vitro* and *in vivo*, is not anchored or supported by an identifiable, validated, and replicated biologically plausible mode of action at levels below AChE depression. This is also a critical point to the human relevance framework and whether results in animal studies can and should be extrapolated to humans. The 2008 SAP Panel concluded the following relative to this question (2008 SAP Minutes at page 28): *"There was a consensus of the Panel that available data were inadequate to support a weight of evidence evaluation for non-cholinergic mode(s) of action for the behavioral alterations following gestational and early postnatal exposure to chlorpyrifos that persisted into adulthood. The Panel agreed that the available information does not allow for behavioral endpoints to be considered as a point of departure and recommended, based upon currently available data, that cholinesterase inhibition be used as the PoD." It should be noted that approximately half of those studies cited in CA DPR Table 14 did not measure cholinesterase inhibition and therefore one cannot infer that the reported findings in these studies were manifested at a level below which ChEI occurs.* From the 2012 SAP (2012 SAP Minutes at page 15), the Panel stated that: *"[T]he number of available neurobehavioral studies is limited leading to caution concerning this finding. Also, many of these studies are statistically under-powered and prone to Type I errors and should be discounted in formulating the weight of evidence for or against neurobehavioral effects from developmental exposure to chlorpyrifos. The Panel also expressed caution with the significance of some of the experimental neurotoxicological outcomes that have not been validated. These included tests of anxiety, depression, and social interactions. The Panel recommends these experimental outcomes be regarded as exploratory, and hypothesis-generating, as opposed to being evidence of toxicity. The lack of specificity in the direction of the neurobehavioral dose response findings is a problematic issue."*

HHA Response: HHA agrees that the area of neurobehavioral/neurodevelopmental toxicity currently has no known MOA or AOP. Note that most of the non-zebrafish *in vivo* animal studies used corn, cottonseed, or peanut oil as the vehicle rather than DMSO. The studies where CPF was administered subcutaneously with a DMSO vehicle were evaluated as part of the Toxicology Profile, but were not emphasized in the PoD discussions. We are aware that subcutaneous dosing is not a likely route of administration. The DMSO effect does not, however, apply to zebrafish for concentrations used in that model. DMSO in zebrafish models is generally used to help absorb the administered chemicals.

18. RCD Page 57:

The Toxicity Forecaster (ToxCast^{TM}) program was launched by the U.S.EPA in 2007 as part of the "Toxicity Testing in the 21st Century (Tox21)" Federal program in collaboration with the National Toxicology Program at the National Institute of Environmental Health Sciences, the National Institutes of Health's National Center for

Advancing Translational Sciences and the Food and Drug Administration (http://www.epa.gov/chemical-research/toxicity-forecasting; accessed 12-2015). ToxCast was designed to prioritize chemicals based on the results of highthroughput screening (HTS) assays indicating potential disruption of key biological pathways. Chemicals were selected for screening by the U.S.EPA (ToxCast) and the Tox21 collaborators, as well as international programs (OECD) and other stakeholder groups. Currently the multiphase ToxCast program, with over 700 unique assays and 300 signaling pathways, has evaluated numerous chemicals (~2,000) with established or unknown toxicity, including cosmetics, drugs, pesticides, and environmental contaminants (Tice et al. 2013). The ToxCast data may be used to elucidate biochemical mechanisms as well as common pathways for human disease outcomes. Ultimately a goal of this U.S.EPA program is to use the ToxCast hazard and exposure data predicted by computer modeling to facilitate chemical risk assessments and prioritization.

DAS Response: The RCD included data from seven ToxCast assay platforms that reported active results for CPF and CPF-oxon ("actives"): ACEA Biosciences, Inc. (ACEA), Apredica (APR), Attagene (ATG), Bioseek (BSK), CEETOC (Cyprotex), CellzDirect (CLD), Novascreen (NVS) and Odyssey Thera (OT), the NIH Chemical Genomics Center (NCGC or Tox21). The assays that the Agency deemed as "true actives" (assays that were not within the range of cytotoxicity) showed generalized activities that were not specific to AChE or neurotoxicity. Other technical comments DAS recommends for CA DPR consideration follow: Clicking one of the links to the data provided in the Agency's draft (http://actor.epa.gov/dashboard2/) did not provide access to the data. Clicking the link to the data provided in the currently active link to the ToxCast data (http://actor.epa.gov/dashboard/) proved that spot-checking of individual AC50 values provided values close to but not identical with those in the report *Chlorpyrifos RCD: Draft 12-31-2015.* The histogram, shown in Figure 7 (pg. 61-62) illustrates the active (true actives + actives: red) and inactive (blue) CPF and CPF-oxon assays along with their intended target families. The histogram should be reconstructed to only focus on those data deemed by the Agency to be "truly active" (i.e., above the cytotoxicity burst) versus the inactive assays. This is consistent with verbiage in the draft report that states "the 'burst region' represents a grey area where true chemical-receptor interactions and assay interference due to cytotoxicity/apoptosis may result in a false positive response". The value derived by the Agency as the cut-off concentration for *Burst Activity* was the same across all of the assays; however, it is likely that individual assays had inherently different "noise" associated with their *Burst*. The finding that many of the assays deemed by the Agency as *True Actives* corresponded to generalized activities that were not specific to AChE or neurotoxicity suggests these might have been secondary to non-specific basal cytotoxicity occurring within that model system but above the Agency's specified Burst cut-off value. For estrogen, androgen and thyroid receptor pathways, both chlorpyrifos and chlorpyrifos oxon compounds were only active within the burst region. This finding contradicts the Agency's indication of potential for endocrine disruption from CPF exposure at higher doses, thus, no relationship of these data to endocrine activity should be made. Moreover, chlorpyrifos has been thoroughly evaluated through the EPA EDSP program and is considered not be endocrine-active for any endpoint evaluated. No further testing by the EPA was recommended. Visually, the

Toxicological Priority Index (ToxPi) was intended to represent a weighted combination of relevant data as component slices of a unit circle, with each slice representing one piece of information. The ToxPi components in Figure 9 (pg. 65-66) of the Agency's draft report included any actives as defined on the ToxCast Website but was not broken down for true actives. The ToxPi graphs should be reconstructed to only focus on those data deemed by the Agency to be "truly active" (i.e., above the cytotoxicity burst) versus the inactive assays. This is consistent with verbiage in the draft report that states "the 'burst region' represents a grey area where true chemical-receptor interactions and assay interference due to cytotoxicity/apoptosis may result in a false positive response".

HHA Response: Thank you for ToxCast link correction. The dashboard data are constantly updated and the results changed accordingly. Only actives were used for the ToxPi calculations. If there are contradictions in the ToxCast findings with the estrogen, androgen, or thyroid receptors, it is likely because CPF needs metabolic activation. This section has been revised since December 31, 2015 with additional information provided by discussions with the US EPA computational toxicology team and through evaluation of new publications (Browne et al. 2015; Judson et al. 2016).

19. RCD Page 66:

Zebrafish (ZF: Danio rerio) provide a model for studying effects of CPF in vivo. They share many developmental, anatomical, and physiological characteristics with mammals since molecular signaling is conserved across species (Padilla et al. 2012; Padilla et al. 2011; Sipes et al. 2011; Tanguay 2013; Tanguay Chlorpyrifos RCD: Draft 12-31-2015 67 et al. 2013). They also require AChE for normal neurodevelopment (Behra et al. 2002a). For that reason, ZF are useful for studies of neurobehavioral developmental effects of AChE inhibitors like CPF. ZF embryos can reveal acute toxic effects of CPF since growth and development occur at such a rapid rate. Therefore, if a chemical is developmentally toxic in ZF, it would affect molecular pathways or processes that might be detected by phenotypic and/or neurobehavioral responses. These changes can then serve as indicators of affected pathways for target identification (Padilla et al. 2012; Padilla et al. 2011; Tanguay et al. 2013; Truong et al. 2014). The two primary models in ZF consist of using either intact embryos (Padilla et al. 2012) or using embryos with the chorion removed (Tanguay et al. 2013) (http://actor.epa.gov/dashboard2/).

DAS Response: DAS provides the following general comments on the use of zebrafish (ZR) as a model for mammalian toxicology, followed by some perspective on the use of the two primary models in ZF, intact embryos (Padilla et al) or embryos with chorion removed (Tanguay et al), and finally offer some comments on study design and confounding variables that accompany many of the studies cited by the CA DPR.

***General Comments*:**
Tests employing treatment of zebrafish embryos in culture dishes are inherently in vitro models. An important issue with extrapolating results from these studies to rodent or human data is that exposure occurs in a closed system. Another critical aspect regarding this closed

system exposure is the dosing kinetics. The zebrafish in this model are continually being bathed in chlorpyrifos from very early in embryonic life through to their larval developmental stages. This is not representative of a mammalian exposure paradigm in the real world where exposure occurs in non-continuous and discrete periods to the mother or the young child. If the chlorpyrifos exposure occurs during pregnancy or during the nursing period, toxicokinetics of the mother (absorption, distribution, metabolism, excretion) need to be factored in before any exposure could even occur to the offspring. The *in utero* environment in mammals is uniquely different from that of fish with additional fluid compartments which impart additional toxicokinetic factors which would not be represented in the in vitro zebrafish embryo testing. Zebrafish have been well studied on the global genomic level for their similarities to mammalian species. It is very common to have gene duplications in zebrafish where only one gene would be represented in mammalians. Non-genomic differences are less well characterized in zebrafish and therefore it is much less clear how effects in zebrafish can be relatable to effects in mammalians. Finally, regulatory testing guidelines are available for mammalian testing for developmental toxicity and neurotoxicity. These include validated and standardized protocols for testing. Clear criteria are therefore available with which to separate excessively toxic test levels, which would invalidate high dose testing where spurious findings might occur, from dose levels which would be considered to be below a maximum tolerated dose. No such standardized or validated criteria are available for similar testing in zebrafish. It has been established that administration of DMSO decreases the barrier function of the zebrafish chorion. Specifically, DMSO concentrations of 0.1% and 1% altered the uptake and distribution of a fluorescent marker in the zebrafish embryo, whereas a concentration of 0.01% DMSO had no effect on uptake (Kais et al., 2013). This supports the current OECD recommendation that a maximum solvent concentration of 100 mg/L (equivalent to 0.01%) in test systems should not be exceeded (OECD, 2000). If used at concentrations ≥0.1% DMSO could increase the availability of co-administered chemicals inside the chorion, but other molecular features of the chemistry (*e.g.* molecular bulkiness) would also be important for determining the amount of uptake and distribution into the fish embryo. DAS notes that a review of many of the studies cited by CA DPR in its RCD on ZF used DMSO as a carrier solvent in excess of the OECD-recommended limit of 0.01%. Use of DMSO (or other solvents) to deliver test chemicals and facilitate uptake across the embryonic fish chorion may be useful in studies where the focus is on maximizing uptake and determining toxicological modes of action. However, co-administration of solvents or other steps taken to compromise or remove the chorion barrier are not relevant for predicting chemical exposure to embryonic fish in the wild, since the chorion would be expected to remain intact in the natural setting. Toxicity testing beyond the embryonic stage to the eleutheroembryo stage (i.e. the stage between hatching and start of intake of external feed) has been recommended as a possible approach to ensure that the most relevant and sensitive fish life stages are evaluated (Embry et al., 2010).

Comments on Padilla et al. publications: The Authors use of “terata” is not consistent with the field of teratology and it would be more appropriate to indicate the effects score as a toxicity score. Malformations (teratogenicity) is one manifestation of developmental toxicity: the others being death, growth retardation, and a functional deficit. The response with CPF-

oxon is typical of what is seen with non-teratogenic compounds in zebrafish embryos where there is a very steep, and often bimodal, response curve from normal embryos to lethality. It is well known that the zebrafish embryo has minimal metabolic capability before day 3 post fertilization (dpf) and by this point most of the embryo development has completed (Jones et al., 2010). This strongly suggests that little metabolism from CPF to CPF-oxon would occur during in this test system before 3 dpf. This is well reflected by the data in that the AC50 response with CPF is > 21x lower than that of CPF-oxon indicating that there is clear difference between the biological action of the two compounds. This contrasts from the statement in this section purporting that the CPF and CPF-oxon have the same terata score (which they do not) which is used to infer metabolism of CPF to CPF-oxon. **In conclusion the zebrafish embryo model is not appropriate for evaluation of CPF effects on early neurodevelopment in relation to humans (emphasis DAS).**

Comments on Tanguay publications: In its assessment of these ZF data, the CA DPR authors suggest that the lack of effects by CPF in this model is due to a difference in the zebrafish's ability to metabolize CPF to sufficient levels of the oxon. This is not a plausible argument as there in no significant difference between the type of zebrafish embryos used in the Padilla research compared to the Tanguay research. Furthermore, see the point mentioned above on the lack of metabolism in early embryos. Additionally, the CA DPR authors also suggest that is it possible in the Tanguay research that CPF is not getting into the embryos. This is very unlikely since dechorionated embryos generally have > sensitivity to chemicals than embryos with intact chorions (as in the Padilla publications). Furthermore, the Tanguay testing used a higher concentration of DMSO than the Padilla research which would only serve to increase the exposure rather than decrease it. The zebrafish (ZF) publications cited in the CA DPR Chlorpyrifos risk characterization document do not adequately support the summary statement, "*Persistent effects from hatching to adults included a decline in ZF brain dopamine and norepinephrine levels, decreased habituation to startle, increased startle response, decreased escape diving response, increased swimming activity and lower learning rate. CPF affected anxiety-related behaviors in ZF (decreased swim speed and thigmotaxis [edge preference/anxiety]). The active concentration of CPF on AChE inhibition in ZF was 0.1 μM. At concentrations not inhibiting AChE (i.e., 0.01μM), CPF caused significant increase in abnormal behavioral (increased "fish at rest", decreased swim speed, decrease in fish with a preference for being on the side or on the edge of their swim lane). At 10-fold lower CPF concentrations than those inhibiting AChE, ZF behaviors were affected during embryonic development.*" Examination of the methods from the individual studies call into question their validity due to flaws in the study design including unknown solvent concentrations, no solvent controls, inadequate replication, de-chorionation, and a lack of analytical confirmation of the exposure concentrations. Relevance of the concentrations tested is also a significant concern. For example, Jin et al (2015) stated that chlorpyrifos inhibited AChE protein levels at ≥ 100 μg/L (0.3 μM) and decreased locomotion (distance and speed) only at a concentration 3X that amount; however since no solvent controls were used nor was the amount of solvent disclosed, one cannot attribute the effects solely to the test material. A review of the methods and results for each study can be found in the table below. More evidence is needed to justify a 10X safety factor for perceived behavioral effects below AChE inhibition levels.

HHA Response: HHA agrees that the zebrafish model has some drawbacks and is not perfectly aligned with mammals. However, it can be a useful model for studying neurobehavioral and neurodevelopmental toxicity. As such, HHA utilizes the zebrafish data in its weight-of-evidence assessment. We noted earlier that 0.1% DMSO does not affect zebrafish toxicity (Maes et al., 2012). OECD has different guidelines for DMSO than US EPA. In addition, DMSO concentrations of 0.64% and 0.4% used by Tanguay et al., and Padilla et al., in the ToxCast zebrafish assays have been endorsed by US EPA. Since the zebrafish assay methods were thoroughly vetted prior to performing the definitive assays, we cannot reject the data simply because a DMSO vehicle was used. We acknowledge our error for the terata scores for CPF (not applicable) and CPF-oxon (40). However, both compounds reach a terata score of 40 in the concentration response. This has been further clarified in the RCD. The AC_{50}s are 8.5 and 0.4 µM for CPF and CPF-oxon, respectively. The zebrafish model is useful for screening, but also provides valuable information on development. The metabolic capacity of the embryos will change without a chorion. The study by Ballough et al. (2008) was an extreme situation where high doses of DMSO were used with high doses of Soman injected intraperitoneally in rats. This study is not representative of typical results or utility of the zebrafish models. The zebrafish section has been revised to reflect DAS comments.

On a separate note, we maintain that it is appropriate to retain the 10x uncertainty factor for neurobehavioral/neurodevelopmental toxicity in developing mammals.

20. RCD Page 74:

> ***"Male and female rats were treated with CPF in an aerosol (nose only) in a single exposure and showed plasma, RBC and lung AChE inhibition (BMDL10 = mg/m3; 0.09 ppm or 0.89 mg/kg/d: Hotchkiss et al., 2010)".***
>
> **DAS Response:** It is not clear why the BMDL value is given after the opening sentence since it would be more appropriate to augment this sentence with the degree of cholinesterase inhibition.

HHA Response: Thank you for the suggestion. The sentence has been revised.

21. RCD Page 74:

> ***"The study of greatest interest for risk assessment is the one performed with aerosol, since that is the most likely media form for human inhalation exposure in California (Kwok, 2015; APPENDIX 3)."*** **Based on the report by Kwok (2015) the CA DPR assumes that aerosol exposure from spray drift is the most likely human exposure scenario in California. DAS commissioned a 6 hour nose only chlorpyrifos aerosol exposure study (Hotchkiss et al, 2010) to provide toxicokinetic data to extend CPF PBPK/PD modeling efforts (Timchalk, 2002; Poet et al., 2014) to include inhalation as a route of exposure. It has been determined that acute 6 hour (Hotchkiss et al, 2013) and repeated subchronic**

(Newton, 1988) exposures of rats to a saturated vapor atmosphere of chlorpyrifos have no measurable effect on ChE activity in any tissue. The use of a solid aerosol of chlorpyrifos was necessary to achieve an absorbed dose sufficient to induce detectable ChE inhibition following a single 6 hour exposure (Hotchkiss et al, 2010). The particulate nature of the chlorpyrifos aerosol used in the Hotchkiss et al (2010) study was unique and is not representative of the composition of spray drift aerosols that might be encountered in the ambient environment. Chlorpyrifos is never applied in its pure state, rather it is formulated with inert ingredients for spray/fog applications, reducing the actual chlorpyrifos concentration in spray drift aerosols relative to the solid aerosols used by Hotchkiss et al (2010). The US EPA has published PoDs for steady-state inhalation exposure for two critical subpopulations (children 1-2 years old: 2.37 mg/m3; and females 13-49 years old: 6.15 mg/m3; US EPA 2014a) that are used for both acute and subchronic CPF spray-drift exposures in California. The PoDs were derived based on the assumption that the regulated aerosol is pure CPF, as used in the Hotchkiss et al (2010) study, when in reality any spray drift aerosol will contain a much lower concentration of CPF, based on the % CPF composition of the sprayed formulation. As such the PoD aerosol concentrations are likely to overly conservative, not reflective of the actual exposure scenario, and should be adjusted for the % CPF in the formulation associated with a spray drift event to yield a comparable inhaled dose of CPF. This would effectively increase, and more accurately reflect, the PoD aerosol concentration in proportion to the % CPF in the spray drift aerosol.

HHA Response: We maintain that aerosols are the most likely media for human inhalation exposure in California.

22. RCD Page 74:

"The U.S.EPA did not anticipate acute inhalation exposure for their residential scenarios. They instead generated PoDs for steady-state inhalation exposure for two critical subpopulations (children 1-2 years-old: 0.00237 mg/m3; females 13-49 years-old: 0.00615 mg/m3) (U.S. EPA 2014a)."

DAS Response: CA DPR has incorrectly stated the PoDs for the two populations (children 1-2 years old and females 13-49 years old). An examination of Table 4.8.4 on page 65 of the USEPA's Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review clearly shows that the PoDs corresponding to 10% RBC inhibition in these two populations are 2.37 and 6.15 mg/m3, respectively, not 0.00237 mg/m3 or 0.00615 mg/m3 as CA DPR states. There is a 1000X difference in the CA DPR-values from what was published by the USEPA.

HHA Response: This has been corrected in the RCD.

23. RCD Page 123:

"CPF affects several neurotransmitters in the CNS that are critical to behaviors related to mood, emotion, learning and memory including the endocannabinoids (Carr et al., 2011, 2013, 2014, 2015), dopamine (Mohammed et al., 2015) and serotonin (Aldridge et al., 2003, 2004, 2005a, b). CPF has been shown to affect behavior related to anxiety in animals (Carr et al., 2015), that is associated with dopamine (Mohammed et al., 2015) and serotonin levels (Aldridge et al. 2005a; Aldridge et al. 2005c; Aldridge et al. 2003; Aldridge et al. 2004). Effects on mammalian neurotransmitters from CPF treatment are presented below. These data show evidence that neurotoxicity may be occurring at doses lower than those causing AChE inhibition and provide evidence of additional MOAs for CPF neurotoxicity."

DAS Response: DAS believes there is insufficient evidence and absence of a defined MOA to support the contention that chlorpyrifos is associated with neurotoxicity below the threshold for cholinesterase inhibition. Numerous independent reviews (including SAP reviews) have evaluated the body of data and purported studies/evidence that associate chlorpyrifos exposure with neurodevelopmental effects and there is consistency in the conclusion across these that protection against cholinesterase inhibition is protective of all other toxicities, including neurodevelopmental effects. Therefore, as the EPA notes in the RHHRA, acetyl-cholinesterase (AChE) inhibition remains the most robust quantitative dose response effect and thus continues to be the appropriate endpoint for use in quantitative risk assessment. As has been recently stated by the USEPA in its RHHRA: "*Overall, across the literature on neurodevelopmental outcomes and including the most recent publications, there continue to be inconsistencies in effects in relation to functional domains, dosing paradigms, and gender-specificity. The only studies reporting effects use doses that inhibit fetal/pup brain activity to some degree, even though there are also negative effects at the same doses. The broad profile of neurological effects that have been reported do not aid in the development of a specific AOP (AChE inhibition or other mechanisms), and existing experimental studies have not been designed to examine and track possible mechanisms from early initiating event to the final neurological outcome."* The Agency goes on to state that "*Overall, a definitive mode of action or adverse outcome pathway leading to effects on the developing brain cannot yet be established because of insufficient data establishing the causal linkages among different levels of biological organization to adversity*." Additionally, the Agency has stated in the RHHRA that "*The SAP concurred with the Agency in 2008 and 2012 about the lack of definable key events in a MOA/AOP leading to neurobehavioral effects. The Agency has considered the new literature since the 2012 SAP related to mechanistic hypotheses as described below (Appendix 11), and note that such a MOA/AOP still cannot be established*." As noted earlier, the EPA convened an entire SAP in 2012 on this question pertaining to experimental studies purporting to associate chlorpyrifos with neurodevelopmental outcomes at exposures below the threshold for cholinesterase inhibition and has commented as follows:
Question 1.0: "…Please comment on the Agency's preliminary conclusion that AChE data remain the most robust source of data for deriving points of departure for chlorpyrifos…"
SAP Response: "The Panel concurs with the Agency's position that AChE data continue to be the strongest resource of data for deriving points of departure for chlorpyrifos….The Panel additionally notes that studies evaluating neurodevelopmental effects entailed

experimental designs that do not permit an efficient means of determining point of departure for chlorpyrifos…Also in keeping with the 2008 SAP, this Panel expresses concern about the use of Dimethyl Sulfoxide (DMSO) as a vehicle…"

Question 3.1: "…Please comment on the degree to which these studies (i.e., experimental toxicology data in laboratory rodents showing neurobehavioral effects) show changes in a number of neurological domains and support the qualitative conclusion that chlorpyrifos exposure during gestation and/or early post-natal period may result in long—term adverse effects on the developing nervous system."

SAP Response: "The Panel agrees with the 2008 SAP conclusions that developmental neurobehavioral studies demonstrate adverse effects from chlorpyrifos exposure. However, the number of available neurobehavioral studies is limited leading to caution concerning this finding. Also many of these studies are statistically under-powered and prone to Type I errors and should be discounted in formulating the weight of evidence for or against neurobehavioral effects from developmental exposure to chlorpyrifos. The Panel also expressed caution with the significance of some of the experimental neurotoxicological outcomes that have not been validated. These included the tests of anxiety, depression, and social interactions. The Panel recommends these experimental outcomes be regarded as exploratory, and hypothesis-generating, as opposed to being evidence of toxicity…Despite the issues raised by the Panel about these studies, the overall evidence across these studies is persuasive in indicating that there are enduring effects on the Central Nervous System (CNS) from chlorpyrifos exposure at or above 1.0 mg/kg/day." Notably, this dose is greater than the NOEL for AChE inhibition at which chlorpyrifos is currently regulated. Given the challenges relative to interpretation of many of the studies that have been published in recent years suggesting non-cholinergic effects from exposure, it is helpful to review the findings of the 2008 SAP reviews relative to conclusions on neurodevelopment related to chlorpyrifos exposure. The SAP concluded that gestational or early postnatal exposures can lead to neurochemical or behavioral alterations that persist into adulthood, although they noted that the studies reporting such effects must be considered in the context of exposure, experimental design, and other influencing variables, the very point DAS has presented earlier in these comments. Specifically, the SAP recommended that inhibition of cholinesterase be used as a point of departure until a mode of action is identified and validated for other putative endpoints or toxicological targets. Related to this, the SAP noted that the majority of these studies have been conducted at or above 1 mg/kg, a sufficient exposure for the inhibition of cholinesterase. The SAP recommended general collaborative efforts to determine if enzyme inhibition is occurring at discrete brain sites at critical periods of development in animals. In addition to the SAP reviews, other independent reviews (examples, Eaton et al. 2008; Li et al. 2012) of the experimental toxicological literature are available which demonstrate that there is more inconsistency than consistency in reported findings and importantly, that there is profound influence impacting reported effects owing to a range of exposure periods, dosing scenarios, testing strategies and specific methodologies and equipment used. In their review, Li et al. (2012) concluded that "there is strong evidence from the animal literature that AChE inhibition (RBC or brain from adult or offspring) is a sensitive endpoint that is protective of neurobehavioral, neuropharmacologic, and morphologic alterations that were measured following gestational, lactational, and/or early postnatal exposure to 1 to 6 mg/kg-d." In reviewing much of the same literature, Eaton et al. (2008) concluded that most of the

in vivo animal studies that report neurodevelopmental and/or behavioral effects occurred in the presence of brain and/or plasma cholinesterase inhibition, while the *in vitro* studies report effects at concentrations that exceed *in vivo* study exposures. Current global regulatory standards for chlorpyrifos are established to limit human exposure to levels well below those causing RCB AChE inhibition. As such, many of the experimental *in vivo* studies have no immediate relevance to humans. Additionally, a few studies report effects on neuronal differentiation at levels below those associated with cholinesterase inhibition, but these still exceed human exposures. Eaton et al. (2008) summarized their review of this area stating that the weight of evidence from animal and *in vitro* studies suggest that neurodevelopment effects are secondary to cholinesterase inhibition.

A recent hypothesis-based weight-of-evidence evaluation (Prueitt et al. 2012) of the neurodevelopmental effects of chlorpyrifos has been published and concluded that a causal association between chlorpyrifos exposure and neurodevelopmental effects in the absence of cholinesterase inhibition in the brain is not plausible in humans, and that the few associations observed in epidemiology studies are most likely attributable to alternative explanations. In summary of this discussion on putative non-cholinergic mechanisms from experimental studies, many of the studies that have reported non-cholinergic effects associated with neurodevelopmental effects were not designed for regulatory decision-making or risk assessment purposes. In addition, specific hypotheses evaluating potential non-cholinergic mode(s) of action have not been adequately proposed, tested, or validated in appropriate animal models.

HHA Response: HHA maintains that there is a growing body of evidence for neurobehavioral and neurodevelopmental effects at CPF levels below those that result in RBC AChE inhibition. The lack of a clear-cut MOA does not negate results from numerous recent studies. While Prueitt et al. (2011) contend that neurodevelopmental effects in the absence of CPF inhibition in the brain is not a plausible mechanism in humans, many reports published since 2012 support the opposite conclusion.

24. RCD Page 129:

Taken together, the ZF, rodent, and human data provide strong weight-of-evidence for the ability of CPF to cause irreversible developmental toxicity, behavior alterations, and metabolic enzyme alterations at very low doses (10x lower than those that cause AChE inhibition in ZF). Although ZF are not mammals, common genes for similar gene function (e.g., AChE) have been conserved across species (Linney et al. 2004); hence the results in this model support the hypothesis that neurobehavioral toxicity initiated in embryos is insidious and permanent at low concentrations of CPF. These studies provide strong weight-of-evidence for the ability of CPF to cause neurodevelopmental toxicity related to learning/cognition/behavior at doses 10x lower than those that cause AChE inhibition that would lead to neuromuscular effects in ZF (0.01 vs. 0.10 uM).

DAS Response: DAS categorically disagrees with this broad, generalized statement (and we would note that this is framed as a hypothesis, which has yet to be tested and verified) that purports to link studies of various quality, not conducted under rigorous globally recognized

regulatory guidelines, and not yet anchored by an identified and verified mode of action (in ZF, animals, humans) particularly at dose levels 10X below the threshold for cholinesterase inhibition. CA DPR fails to bring into the analysis any weight of evidence process such as the EPA's Draft Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment (Prueitt et al., 2012). For humans, the contention is that lower working memory decrements in children at 7 - 11 years of age are associated with prenatal chlorpyrifos exposure and accompanying this assumption is that prenatal exposures in the Columbia University study (Rauh et al., 2006) were lower than that associated with AChE inhibition. DAS disagrees with the premise that deficits neurobehavioral deficits in children were due to chlorpyrifos exposure and therefore disagrees with the contention that evidence exists in humans to support neurodevelopmental deficits below the threshold where AChEI occurs. For animals, there is no compelling or consistent evidence to support the contention that neurodevelopmental outcomes occur at exposures 3-10X below where AChEI occurs. In many of these studies, cholinesterase has not, in fact, been measured. Most of the studies have employed doses (> 1 mg/kg/day) that are certainly associated with RBC cholinesterase inhibition and this is the conservative (protective against brain ChEI) endpoint upon which regulatory bodies globally base human exposure limits. In the case of the ZF studies and evidence that has been brought forward, DAS has earlier commented that there are design flaws and methodological confounders (use of DMSO as a solvent carrier) that prevent this line of evidence from supporting the contention that chlorpyrifos in ZF is causally linked to neurobehavioral toxicity, particularly at exposures 3-10X below where ChEI occurs. In fact, in many of the studies cited, ChEI was not measured and in some cases where it was, inhibition was reported, thus, contradicting the overarching statement about a causal link at low chlorpyrifos exposures.

HHA Response: HHA values the zebrafish model for weight-of-evidence in the CPF risk assessment.

25. RCD Page 130:

"An UF of 10 for intraspecies variability for oral, dermal and inhalation exposure was based on physiological changes (e.g., AChE fluctuations) in women during pregnancy (U.S. EPA 2014a). This intraspecies variability in the UF also pertains to male and female infants, children and youths since the data used by Smith et al. (2014) to model age-related variability (age 6 months to >16 years) used few samples (30 hepatic microsome, 20 plasma samples) to estimate intraindividual age-related variability of PON1 and cytochrome P-450 enzyme activity for all subpopulation groups (including variability representing all ethnic populations). Different ethnic populations demonstrate vastly different PON1 activities (Diepgen and Geldmacher-von Mallinkrodt 1986) and P450 phenotypes, factors that can influence CPF toxicity. Of the 120 parameters in the CPF PBPK-PD model only 16 were used for variability in the Data Derived Extrapolation Factor (DDEF) intra-species analysis. Only four of the 16 parameters were used to drive more than 80% of the RBC AChE inhibition (hepatic P450 metabolism of CPF → CPFoxon, hepatic P450 detoxification of CPF-oxon → TCPy; hepatic PON1 detoxification of CPFoxon → TCPy, plasma PON1 detoxification of CPF-oxon → TCPy) (U.S. EPA 2014a). The variations are

due to genotypic and phenotypic differences which affect and the rates of detoxification and activation in humans (Berkowitz et al. 2004; Diepgen and Geldmacher-von Mallinkrodt 1986; Furlong et al. 2006). CPF was found in 70.5% of pregnant mothers living in the Salinas Valley in California (Huen et al. 2010) putting both fetuses, that cannot metabolize OP, as well as their mothers, at risk (Chen et al. 1999; Furlong et al. 2006). Of concern as well is the uncertainty that autopsied tissues used for input data may or may not produce the relevant enzyme activities (i.e. plasma PON1, hepatic PON1, hepatic P450 bioactivation to oxon and hepatic P450 detoxification to TCPy) resembling normal human microsomal or plasma enzymes, even though the PBPK-PD model is designed to compensate for their potential differences (Poet 2015; Smith et al. 2011). Various uncontrolled processes of autolysis and degradation along with inconsistent quality of tissues can ultimately affect the interpretation of data derived from them. Therefore the UF of 10 is used to account for intraspecies variability related to age, inter- and intra-ethnic differences in enzyme activities (e.g., PON1 and P450) and genotypic frequencies in populations that have greater susceptibility to CPF toxicity (Eaton et al. 2008; Jarvik et al. 2003).”

DAS Response: DAS agrees that any major loss in enzyme activity during tissue sample collection, processing or storage could impact the utility of using *in vitro*-derived metabolic rates for *in vivo* blood level predictions. However, in the case of PON1 enzyme levels in plasma or liver microsomal tissue samples from human donors, there is excellent data to show that this enzyme is quite stable during sample collection and storage. Huen et al. (2009) conducted a longitudinal study to evaluate the storage stability of PON1 activity in human plasma samples (n=95). These authors found no change in PON1 hydrolysis activity for the chlorpyrifos oxon after 2 years of storage at -80˚C. Extended storage out to 7 years also resulted in less than 40% loss in plasma PON1 activity. In a similar manner, Gonzalvo et al. (1998) studied the stability of PON1 enzyme activity in liver tissue isolated from rats that were sacrificed and livers allowed to remain in the body at room temperature (25 ˚C) for up to 24 hours prior to dissection and processing to microsomes. These authors found that at 3, 6, 12 and 24 hours post-sacrifice the PON1 activity remained at 83%, 83%, 73% and 51%, respectively, of the levels from liver tissue processed immediately after sacrifice to microsomes. The overall results of these stability experiments show that PON1 activity is fully stable for 2 years in frozen plasma, with less than a 40% loss after 7 years. PON1 activity is also well retained during an extended tissue collection time, with liver enzyme functionality declining by less than 30% after 12 hours at room temperature. Based on these well-conducted studies, DAS believes that the PON1 enzyme activity levels used in the PBPK model are accurate for predicting the systemic exposure to chlorpyrifos and the oxon metabolite, as well as RBC cholinesterase inhibition in human cohorts, including pregnant women. The PBPK model-derived DDEF of 4 should therefore be an appropriate Uncertainty Factor for intraspecies variation in biological response to chlorpyrifos.

HHA Response: The information provided by DAS has been added to the RCD, however it does not fully address the uncertainties associated with PBPK-model derived DDEF. At this time, HHA is not deviating from the default uncertainty factor of 10 for intraspecies variability.

26. RCD Page 130:

"A further UF of 10 is based on neurodevelopmental and neurobehavioral effects occurring in human fetuses in utero and during development (Hattis 2015; Horton et al. 2012; Lovasi et al. 2011; Perera et al. 2003; Rauh et al. 2011; Rauh et al. 2006; Rauh et al. 2012; Reiss et al. 2015; Whyatt et al. 2009; Whyatt et al. 2007; Whyatt et al. 2004) at exposure levels lower than those inducing RBC, plasma or brain AChE inhibition. Berkowitz et al. (2004) showed an association with PON1 status and head circumference in children exposed to CPF in utero.

DAS Response: A critical analysis of published information from varying scientific disciplines and perspectives reveals that findings …have limitations, including reliability of reported results, exposure to other risk factors, lack of reproducibility of findings in other studies, and incompatibility with the voluminous toxicology database for chlorpyrifos (Eaton et al., 2008; Prueitt et al., 2011; Li et al., 2012; Burns et al., 2013). In fact, the European Food Safety Authority (EFSA) recently concluded in its review on epidemiological studies linking exposure to pesticides and health effects that there is "no evidence" to suggest an association between pesticide exposure and neurodevelopmental related outcomes, due to a number of deficiencies in the available data (Ntzani et al., 2013).

HHA Response: A 10x factor will be retained for uncertainties related to neurobehavioral/ neurodevelopmental toxicity.

27. RCD Page 130:

Data also support the findings of disruptions from CPF in the CNS (serotonergic and endocannabinoid pathways) at exposure levels lower than those inducing brain AChE inhibition in preweaning rats (<0.5 mg/kg/d) that result in neurobehavioral/neurodevelopmental effects (Carr et al. 2015; Carr et al. 2014; Mohammed et al. 2015)."

DAS Response: DAS would emphasize that two (Carr et al., 2015; Mohammed et al., 2015) of the three citations used to support this statement are abstracts only and not full peer-reviewed publications. As such, experimental details needed to evaluate and make a determination on the biological basis for this statement are not available. The abstracts did not include any mention of concomitant measurement of cholinesterase inhibition and thus, it cannot be empirically inferred that brain cholinesterase inhibition did not occur. Additionally, in Carr et al (2014), while experimental data were reported that showed no relative inhibition of brain ChEI, the data did in fact show statistically significant inhibition of serum ChEI, which is the relevant point in that US EPA and other global authorities base human exposure limits on protection against RBC inhibition, not on inhibition of brain cholinesterase. Thus, if the inference is that CPF is associated with biological changes in the CNS, the comparison should be relative to the putative exposure associated with serum or RBC inhibition, not brain ChEI. It is also important to recognize that the lowest administered dose in these studies cited above was 0.5 mg/kg/day, a dose level where RBC ChEI occurs

and one that is above the EPA's point of departure for risk assessment purposes. Thus, while the contention that CNS effects are occurring below where brain ChEI occurs, it is imperative to remember that exposure limits to humans are based on protection against 10% RBC ChEI, which are protective of exposures at which CNS effects may be observed.

HHA Response: The abstract by Carr et al. has been published in Neurotoxicology (2017 Mar; 59:183-190) and the reference was added in the revised RCD. Mohammed et al. 2015 abstract has been accepted for publication.

III. EPIDEMIOLOGY AND UNCERTAINTY FACTORS

1. RCD Page 51ff:

II.K. Epidemiology Studies Related to Neurodevelopmental Effects
II.K.1.a. The Columbia University's Mother's and Newborn Cohort (CCCEH Cohort "Columbia Study")

DAS response: The Columbia study is not useful for informing the question of whether neurodevelopmental effects occur at exposure levels lower than those associated with acetylcholinesterase inhibition or for "bounding" dose-response estimates from animal studies.

The Columbia study alone is not sufficiently robust to make a causal inference of any given health effect and chlorpyrifos exposure. In following several hundred children for a more than a decade, the Columbia study has also reported several adverse health associations with prenatal chlorpyrifos exposure as measured in cord blood. The study has also reported varied adverse health associations with exposure to air pollution, bisphenol A, lead, phthalates, polybrominated diphenyl ethers, and second hand smoke (http://ccceh.org/our-research/scientific-papers). A number of limitations of the study have been highlighted in several publications and public comments. Importantly all the Columbia publications are based upon a single spot sample for exposure that was not timed with an application. Further, the analytical method used in the Columbia study has not been validated at the low concentrations reported in maternal/cord blood from Columbia study subjects. Finally, there are credible alternative explanations for the observed effects. Because of these limitations, it is even more important to compare age and outcome specific results of the Columbia study with other epidemiology studies. The following abstract from a public letter to the US EPA summarized these points of considerations of reliability and utility of epidemiology: "*The utility and application of epidemiology data in risk assessment and regulatory decision-making has received considerable attention in recent years and continues to be vetted by the U.S. Environmental Protection Agency (USEPA) when evaluating chemical risk to human populations (e.g., SAP 2010). For the insecticide chlorpyrifos, there exists a growing number of studies that may inform the risk assessment for this chemical, although one cohort investigated by Columbia University is being considered by the USEPA as providing evidence for the relationship between chlorpyrifos exposure and children's development and*

cognitive function (i.e., Rauh et al., 2006, Rauh et al., 2011; Whyatt et al., 2004). A critical analysis of published information from varying scientific disciplines and perspectives reveals that findings from this singular cohort have limitations, including reliability of reported results, exposure to other risk factors, lack of reproducibility of findings in other studies, and incompatibility with the voluminous toxicology database for chlorpyrifos (Eaton et al., 2008; Prueitt et al., 2011; Li et al., 2012; Burns et al., 2013). In fact, University researchers (Ntzani et al. 2013) under contract from the EU's European Food Safety Authority (EFSA) reviewed the epidemiology studies published since 2006. They concluded there is no evidence to suggest an association between pesticide exposure, including chlorpyrifos, and neurodevelopmental effects. This review included neurodevelopment/IQ studies on chlorpyrifos that were published in 2006 and later. The totality of problems relating to the reliability of the reported findings on the Columbia cohort renders the study inappropriate for risk assessment. The study is not useful for informing the question of whether neurodevelopmental effects occur at exposure levels lower than those associated with acetylcholinesterase inhibition or for "bounding" dose-response estimates from animal studies." (Edwards 2013)

HHA Response: While the Columbia Cohort study does not provide dose-response data for quantitative risk assessment, the study is important to the completeness and transparency of the RCD and our efforts to document ongoing epidemiological studies that are investigating associations between potential gestational environmental exposures and health outcomes in offspring later in life. The RCD revision includes more extensive discussion of PON1 and CYP based on information provided in the DAS comments.

2. RCD Page 53-56:

II.K.1.b - e. Chlorpyrifos doses in Columbia study, UC Berkley's CHAMACOS Cohort, the Mount Sinai cohort

DAS response: There is growing evidence that age specific results, such as Working Memory in young children, are not consistently associated with chlorpyrifos across the published studies. PON1 is not a significant predictor of susceptibility to chlorpyrifos exposure and health effects in the published cohorts. The Risk Characterization Document from DPR does not fully review the published epidemiology literature nor does it evaluate age specific endpoints across studies. In section II.K.1.b, the DPR discusses results from the Columbia study of the Working Memory function of the IQ test. The Columbia study investigators reported statistically significant decrement of log transformed Working Memory scores with increasing chlorpyrifos blood levels. Notably significant decrements were not observed for other IQ indices of Verbal Comprehension, Perceptual Reasoning and Processing Speed (Rauh et al. 2011). The UC Berkley CHAMACOS investigators also evaluated IQ scores (Bouchard et al., 2011). Bouchard et al (2011) did not observe a statistically significant association with Working Memory or Full Scale IQ and maternal diethyl phosphate metabolites (ΣDEP). Furthermore, since the CHAMACOS investigators collected both chlorpyrifos in cord blood and urinary TCPy metabolite (3,5,6- trichloro-2-pyridinol) but are not reported by Bouchard et al., (2011), it can only be assumed that no

association was present for these more chlorpyrifos specific exposure estimates (Castorina et al. 2010; Huen et al. 2012). Clearly release of this data would be beneficial to refute or support the correlation between exposure and purported effects in humans. The Mount Sinai investigators also reported no statistically significant association with any IQ function, including Working Memory and ΣDEP or TCPy. Adding to the growing evidence the PELAGIE study of children in France reported no inverse association with Working Memory and ΣDEP (Cartier et al. 2015). The RCD discusses on pages 54 and 55 that PON1192 phenotypes (QQ; QR; RR) can affect organophosphate toxicity and may be used to predict relative sensitivity of humans. The RCD fails to discuss that PON1 does not impact human sensitivity below exposures that do not lead to cholinesterase inhibition. Timchalk et al. (2002) conducted a Monte Carlo analysis of the impact of PON1 phenotypes on brain concentrations of chlorpyrifos oxon, and found at low environmentally relevant doses (5 μg/kg) there is considerably less variability in the estimated brain CPF-oxon AUC between PON1 phenotypes (cv range from 17 to 24%), and more importantly the PBPK model response is relatively insensitive to the variability in CPF-oxonase activity. Sensitivities by PON1 were not observed in an occupationally exposed cohort (Albers et al. 2010). In the CHAMACOS and Mt. Sinai studies, there was no interaction of PON1 phenotype with prenatal urinary ΣDEP metabolite and IQ indices. (Engel et al. 2011; Eskenazi et al. 2014). As a point of clarification, the Mt. Sinai only reported significant associations with the PON1 QQ phenotype for Perceptual Reasoning and ΣDAP and ΣDMP, which are not specific for chlorpyrifos. Further, neither study report on PON1 and IQ with the urinary TCPy metabolite, again suggesting there was no relationship. Finally, it is important to understand that nearly all of the assays for PON1 activity have employed concentrations of oxon substrates in the high μMolar to mMolar ranges. In a critical recent study by Coombes et al. (2014), these authors compared chlorpyrifos-oxon hydrolysis rates at both a high μMolar substrate concentration, as well as a more environmentally relevant mid-nMolar concentration. The authors conclude that "*no significant differences were demonstrated between the PON1192 genotypes and/or between high and low serum PON1 phenotypes at this lower CPO concentration, contrasting with our high CPO concentration data discussed above as well as with studies of others using direct assay methods and high concentrations of CPO (Cole et al., 2005; Jansen et al., 2009; Li et al., 2000; Shih et al., 1998). Therefore, at a low concentration more reflective of levels that would occur under realistic exposure scenarios, neither PON1192 genotypes nor serum PON1 phenotypes influence the capacity of PON1 to metabolize CPO.*"

HHA Response: The RCD has been revised to provide a more comprehensive review of the published epidemiological literature on chlorpyrifos and developmental effects. As mentioned, a discussion of the PON1 and CYP variabilities have been revised in the RCD based on the information in the DAS comments.

3. RCD Pages 56-57:

II.K.1.f. Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural
Pesticides: The CHARGE study

DAS response: The Risk Characterization Document from DPR appropriately lists important weaknesses of this study. The fallacy of using residence and pesticide application as a proxy for valid exposure is fully discussed by Chang et al. (2014). Since actual human exposure is unlikely, the associations with applications of chlorpyrifos (and other pesticides) and cases of autism spectrum disorder are likely due to chance and confounding.

HHA Response: In the absence of true exposure data, the CHARGE Study authors used DPR's Pesticide Use Database and USGS's meridian-township-range-section (MTRS) designations to consider participants' residences, pesticide applications within 1, 1.5, and 1.75 kilometer buffers of the residences, and the overlap of dates of application with the dates of pregnancy, thus developing a proxy exposure profile for each mother. Indeed, there are no biological measures of pesticides or their metabolites, nor any indoor/outdoor sampling that was conducted. However, the CHARGE Study authors were transparent in listing the several potential confounders, including exposure misclassification, lack of data on hours spent at the mapped residence, and a lack of time-based associations of exposure and observed effects. The CHARGE Study has not quantified exposures to pesticides during gestation, although it is important to the completeness of the RCD to document ongoing epidemiological cohorts that are investigating associations between potential gestational environmental exposures and health outcomes in offspring later in life.

4. RCD Pages 126-129:

VI.F. Uncertainties from Human Studies (pages 126 – 129)

DAS response: The available analyses support the EPA point of departure and mechanism of action through cholinesterase inhibition. The uncertainty in the epidemiology studies is perpetuated by not critically reviewing all publications and a lack of transparency of the cohort studies, including lack of access to the actual data.

HHA Response: It is true that not all epidemiological studies investigating adverse health outcomes associated with pesticide use have well-documented or quantified assessment of exposure. And, if the studies do have such data, the raw biomonitoring results are not readily available for additional review. However, as stated above, it is important to the completeness of the RCD to summarize results from ongoing cohorts that are investigating links between potential gestational environmental exposures and health outcomes in offspring later in life. In addition, it is important to weigh potential associations seen in epidemiological studies as important scientific investigations continue into the potential mechanisms of action and adverse outcome pathways for chlorpyrifos and neurodevelopmental toxicity.

5. RCD Page 126-127:

VI.F.1. Columbia Cohort Study

DAS response: The RCD cites limitations and SAP concerns related to the Columbia study. The SAP 2008 conclusion that "chlorpyrifos likely played a role in the observed neurodevelopmental outcomes" conflicts with reviews in the peer reviewed scientific literature that describe the epidemiology evidence as inadequate, inconsistent and biologically implausible (Burns et al. 2013; Eaton et al. 2008; Li et al. 2012; Mink et al. 2012; Needham 2005; Prueitt et al. 2011; Reiss et al. 2015; Zhao et al. 2005). These publications highlight that the results for chlorpyrifos reported by the Columbia investigators must be compared to findings in other studies. As other researchers have noted, it is crucial to conduct quantitative sensitivity analyses when important policy decisions are to be based on the results of epidemiology research (Burns et al. 2014; Christensen et al. 2015; Jurek et al. 2008). This has not been done in the evaluation of the Columbia Cohort study.

HHA Response: In our draft risk assessment we concluded that the available human epidemiological and animal toxicology studies for CPF provide evidence for neurobehavioral effects following developmental exposure to chlorpyrifos. Furthermore, in these studies the neurodevelopmental effects appeared to be as sensitive as or more sensitive than AChE inhibition. However, sufficient data regarding dose-response and the critical time and duration of exposure are presently not available for quantitative use of these data in risk assessment. Therefore, HHA based its current evaluation on RBC AChE inhibition as a critical endpoint and applied a 10 X uncertainty factor for the potential neurodevelopmental effects of CPF.

6. RCD Page 127-128:

VI.F.2. Uncertainties in the PBPK-PD Models Applied to Effects on Working Memory at Age 7.

DAS response: Dow AgroSciences submits that uncertainties in the PBPK-PD models have been addressed or are not relevant. There is poor consistency of the association of chlorpyrifos and Working Memory across four different studies (see above). Further, there is growing evidence that age specific results, such as Working Memory in young children, are not consistently associated with chlorpyrifos across the published studies. It is unclear what DPR has done with exposure estimates below the LOQ without access to the study data. Misclassification of exposure from the single spot sample is a concern that should be recognized by the Department. Incorrect exposure levels create invalid results, not uncertainty. For example, chlorpyrifos elimination rates in exposed humans are fairly rapid, at 27-104 hr (Drevenkar 1993, Nolan 1984, Vasilic 1992). Due to this fairly rapid clearance of chlorpyrifos vs. more slowly eliminated compounds like lead ($t_{1/2}$ = 672-864 hr; ATDSR 2010), the timing between exposures to episodic pesticide applications and biomonitoring measurements could greatly impact the calculation of dose/blood level values. For example, exposures at the end of the second trimester would need to be ~90-fold higher than exposures at the end of the third trimester, depending on exposure scenario, to result in comparable chlorpyrifos blood levels at birth (672 hr/month / 104 hr half-life = 6.5 elimination half-lives per month). Also, the frequency and magnitude of pesticide applications would have a great impact on dose reconstructions of the Columbia study cohort. Unfortunately, these data were

not measured in the Columbia epidemiology study (U.S. EPA 2014, p. 386-391). The investigators were also not able to estimate these data from the study questionnaires, as that data was deemed to be of low quality. Therefore, due to the lack of data on exposure scenarios and the fairly rapid clearance of chlorpyrifos, the method employed by Hattis (2013) of only calculating dose/blood level data from steady-state scenarios underestimates the variation in actual exposures in the Columbia study cohort. Accurate dose reconstruction would require additional data on temporality and magnitude of pesticide exposures, or alternately, simulations of a range of possible residential exposure timelines.

HHA Response: HHA discussed the Hattis model, but did not use it for PoD determination.

7. RCD Page 126-127:

VI.F.3. Discussion of Mt. Sinai Conclusions

DAS Response: This section of 2 sentences looks incomplete. We remind the Department that results from a single epidemiology study should not be promulgated alone without recognizing that other published studies do not validate the observations, in particular head circumference (Mink et al., 2012).

HHA Response: This section has been revised in the RCD.

8. RCD Page 128-129:

VI.F.4. ToxCast and Zebrafish HTS Assays

DAS Response: A vast majority of the experimental studies that are cited as forming the basis for a proposed linkage between chlorpyrifos and effects on neurodevelopment and neurobehavior have methodological/design challenges which severely limit their utility in a weight-of-evidence assessment. These factors include dose, inappropriate route of exposure (i.e. de-chorionation), utility of the neurotoxic vehicle DMSO, lack of solvent controls, inadequate replication, and a lack of analytical confirmation of the exposure concentrations. Collectively, these factors, along with other scientific perspectives and bases commented on earlier (see RCD Page 66 DAS Response) lead DAS to disagree with the DPR statement that "These studies provide strong weight-of-evidence for the ability of CPF to cause neurodevelopmental toxicity related to learning/cognition/behavior at doses 10x lower than those that cause AChE inhibition that would lead to neuromuscular effects in ZF (0.01 vs. 0.10 uM)."

HHA Response: Please see our responses on page 13 and 23 of this document.

IV. DIETARY EXPOSURE

1. DAS COMMENTS

Contrary to the RCD's representation, illegal residues of chlorpyrifos in food are not "frequent" or "high", but rather infrequent, and when [they] occur are primarily associated with illegal uses from imported food.

HHA Response: We disagree with DAS's statement that illegal residues of chlorpyrifos in food are "rather infrequent." Illegal chlorpyrifos residues were detected by both PDP and California Pesticide Residue Monitoring Program (CPRMP). The rate of these detections occurred mostly on commodities for which there is no tolerance established and many were on products not grown in California. For example, for commodities like cilantro and cactus, the detection frequency was 29% and 10%, respectively (218 detected residues/739 cilantro samples tested in PDP for 2009-2012; and 16 detected residues/164 cactus samples tested in CPRMP for 2012-2014; see Table 1 in the RCD and Table 14 in the Appendix 2). As stated in the RCD, HHA does not evaluate illegal residues on agricultural commodities in its dietary exposure assessments and such residues come under the purview of DPR's Enforcement Branch. Nevertheless, the detection of illegal chlorpyrifos residues on commodities sold in California could suggest that the risk from acute dietary exposure to chlorpyrifos may be unaccounted for due to these additional exposures not considered in the current dietary assessment.

2. DAS COMMENTS

DPR state that MOEs for banana and grapefruit are lower than 100 when in fact they are above the target of 100.

HHA Response: We agree with DAS. This statement was an error. Please note that DPR has recently changed its practice and will no longer evaluate the health-protectiveness of the pesticide tolerance for each individual commodity (CDPR 2017). Accordingly, our dietary exposure assessment for chlorpyrifos was revised to remove this section.

3. DAS COMMENTS

The PDP monitoring data obtained from analyzing a large number of commodity samples over a long period of time indicates that it is very unlikely that a consumer would be exposed to a commodity containing residues at or above the tolerance level.

HHA Response: As stated in the RCD and in our response on page 37 of this document, DPR does not evaluate illegal residues on agricultural commodities in its dietary exposure assessments. Therefore, we don't plan to assess the impact of illegal residues on exposure.

4. DAS COMMENTS

The CA DPR acute dietary risk assessment for broccoli, cabbage and orange should be refined using the RDF files available from USEPA.

HHA Response: For the tolerance assessment we used a point estimate (tolerance), not a distribution of residues. Regardless, the revised RCD does not include tolerance-level dietary

exposure assessments as part of overall dietary exposure assessments (DPR, 2017). See our response to DAS comment 2 on page 37 of this document.

V. BYSTANDER EXPOSURE

VI. BYSTANDER RISK

VII. REPORTED INCIDENT DATA

VIII. RISK APPRAISAL

36. RCD Page 121.

See RCD Section ***VI.C. PBPK-PD Model Uncertainties***

DAS Comments: As commented previously in Section II Toxicology, while DPR contends that the PBPK model fails to address physiological changes in pregnancy and therefore recommend a UF of 10X for intraspecies uncertainty, DAS notes that to investigate the appropriateness of the default 10x DDEF, or Intraspecies Uncertainty Factor, for pregnant workers, the current Multi-Route PBPK/PD model was expanded to include systemic exposure and RBC effects predictions during all stages of human pregnancy in April 2015 (Poet 2015). This Pregnancy PBPK model was then used to validate the applicability of the new 4x DDEF for the chlorpyrifos POD in humans to pregnant women as well. Changes were made in physiology in the PBPK model based on the relevance to CPF and CPF-oxon disposition and pharmacodynamics, and using well-established reference values for human pregnancy (Poet 2015, MRID 49635101). Model changes include:

- Addition of placenta and fetal compartments, which grow over the course of pregnancy.
- Pregnancy specific changes in the slow compartment, fat, and rapid compartments.
- Pregnancy specific changes in blood composition
  - Changes in blood composition result in increased blood volume, decreased hematocrit
  - Lipids, triglycerides, and cholesterol increase – leads to changes in partitioning
- Pregnancy specific changes in metabolism
  - CYP450 enzyme levels in liver
  - PON1 activity levels in liver and plasma

These important changes are included in the CPF model for pregnancy, built on the lifestage platform so either age-specific parameters or initial body weight-specific parameters can be used as the initial condition at the beginning of gestation. All model additions, changes, mathematical implementations, and model code are included in the Pregnancy PBPK model report, submitted to the Agency in April 2015 (Poet 2015) and to CA DPR in August 2015. For all simulations in that report, either age was set to 30 years, or a body weight of 69 kg, consistent with US.EPA, 2015 and the Exposure Factors Handbook mean body weight for females (US EPA 2011, Table 8-5). Enzyme activity incorporated into the PBPK model,

across life-stages and in pregnant women, was based on *in vitro* measurements of CYP and PON1 rates in liver tissue and PON1 rates in plasma across a wide age range. Final ranges of enzyme activity used in the model were far wider than the measured values to accommodate a conservative estimate of variation in this critical model parameter across a human population. Also, age-based increases in enzyme ontogenies were included in the PBPK model. **These additional investigations support the adoption of a 4X UF for intraspecies uncertainty** (emphasis DAS).

HHA Response: There remains uncertainty about the use of a single point estimate rather than a distribution for determining the PoD, as well as the small numbers of tissue samples and use of cadaver tissues in the model. However, Section VI.C of the RCD (PBPK-PD Model Uncertainties) has been revised and updated to better reflect our scientific conclusions.

37. RCD Page 123:

DPR contends there is *"…remaining uncertainty and gathering human and animal data related to neurotoxicity during development at doses lower than those inducing the sentinel AChE inhibition…"*

DAS Comments: There is insufficient evidence and absence of a defined MOA to support the contention that chlorpyrifos is associated with neurotoxicity below the threshold for cholinesterase inhibition. Numerous independent reviews (including SAP reviews) have evaluated the body of data and purported studies/evidence that associate chlorpyrifos exposure with neurodevelopmental effects and there is consistency in the conclusion across these that protection against cholinesterase inhibition is protective of all other toxicities, including neurodevelopmental effects. Therefore, as the EPA notes in the RHHRA, acetyl-cholinesterase (AChE) inhibition remains the most robust quantitative dose response effect and thus continues to be the appropriate endpoint for use in quantitative risk assessment. **There is no compelling scientific (animal or human) evidence or a proposed, tested, and validated mode of action to support either the contention that chlorpyrifos is associated with neurodevelopmental effects in humans or that there is any scientific basis for inclusion of a 10X uncertainty factor related to putative neurodevelopmental effects in humans** (emphasis DAS).

HHA Response: As stated earlier in this document, while there is no direct AOP or mechanism associated with neurobehavioral/ neurodevelopmental toxicity, many studies support that such effects occur at doses or exposure levels below those that inhibit 10% RBC AChE.

38. RCD Page 123-124

See Section *VI.D. Uncertainties Factor for Neurodevelopmental Effects*

DAS Comments: A vast majority of the experimental studies that are cited as forming the basis for a proposed linkage between chlorpyrifos and effects on neurodevelopment have methodological/design challenges which severely limit their utility in a weight-of-evidence

assessment. These factors include dose, inappropriate route of exposure and utility of a neurotoxic vehicle, factors which have been highlighted by Scientific Advisory Panels (SAP's) as limiting the utility of reported results when considering relevance to humans. Several SAP reviews in addition to the EPA's Revised Human Health Risk Assessment (RHHRA) are consistent in their finding that a chlorpyrifos mode-of-action/adverse outcome pathway (MOA/AOP inclusive of non-cholinergic mechanisms) leading to neurobehavioral effects cannot be established. This has led to the EPA stating: "*…uncertainties such as the lack of an established MOA/AOP for neurodevelopmental effects and the potential exposure to multiple-AChE inhibiting pesticides preclude definitive causal inference*." Numerous independent reviews (including SAP reviews) have evaluated the body of data and purported studies/evidence that associate chlorpyrifos exposure with neurodevelopmental effects and there is consistency in the conclusion across these that protection against cholinesterase inhibition is protective of all other toxicities, including neurodevelopmental effects. Therefore, AChE inhibition remains the most robust quantitative dose response effect and thus continues to be the appropriate endpoint for use in quantitative risk assessment.

HHA Response: At this time RBC AChE inhibition may provide the most robust quantitative dose response effect. However, this endpoint is only an indicator of exposure. Again, neurodevelopmental and neurobehavioral effects occurring in humans and animals that are associated with CPF exposure at doses lower than those inhibiting 10% RBC AChE cannot be ignored, as would be suggested by use of AChE inhibition, alone.

39. RCD Pages 126-127.

VI.F. Uncertainties from Human Studies

DAS Comments: DAS agrees that there uncertainties from human studies, and as such these studies should be treated with the appropriate level of caution. The available analyses support the EPA point of departure and mechanism of action through cholinesterase inhibition. The uncertainty in the epidemiology studies is perpetuated by not critically reviewing all publications and a lack of transparency of the cohort studies, including lack of access to the actual data. The DPR cites limitations and SAP concerns related to the Columbia study. The SAP 2008 conclusion that "chlorpyrifos likely played a role in the observed neurodevelopmental outcomes" conflicts with reviews in the peer reviewed scientific literature that describe the epidemiology evidence as inadequate, inconsistent and biologically implausible (Burns et al. 2013; Eaton et al. 2008; Li et al. 2012; Mink et al. 2012; Needham 2005; Prueitt et al. 2011; Reiss et al. 2015; Zhao et al. 2005). These publications highlight that the results for chlorpyrifos reported by the Columbia investigators must be compared to findings in other studies. As other researchers have noted, it is crucial to conduct quantitative sensitivity analyses when important policy decisions are to be based on the results of epidemiology research (Burns et al. 2014; Christensen et al. 2015; Jurek et al. 2008).

HHA Response: HHA is treating the epidemiology study data with a great deal of caution while also using it as evidence in support of CPF-initiated neurobehavioral and neurodevelopmental

effects. We do not have PoDs generated from these data, but the results are sufficiently important as to necessitate additional uncertainty factors.

30. RCD Page 127-128.

VI.F.2. Uncertainties in the PBPK-PD Model Applied to Effects on Working Memory at Age 7

DAS Comments: Dow AgroSciences submits that uncertainties in the PBPK-PD models have been addressed or are not relevant. There is poor consistency of the association of chlorpyrifos and Working Memory across four different studies (see above). Further, there is growing evidence that age specific results, such as Working Memory in young children, are not consistently associated with chlorpyrifos across the published studies. It is unclear what the DPR has done with exposure estimates below the LOQ without access to the study data. Misclassification of exposure from the single spot sample is a concern that should be recognized by the DPR. Incorrect exposure levels create invalid results, not uncertainty. For example, chlorpyrifos elimination rates in exposed humans are fairly rapid, at 27-104 hr (Drevenkar 1993, Nolan 1984, Vasilic 1992). Due to this fairly rapid clearance of chlorpyrifos vs. more slowly eliminated compounds like lead ($t_{1/2}$ = 672-864 hr; ATDSR 2010), the timing between exposures to episodic pesticide applications and biomonitoring measurements could greatly impact the calculation of dose/blood level values. For example, exposures at the end of the second trimester would need to be ~90-fold higher than exposures at the end of the third trimester, depending on exposure scenario, to result in comparable chlorpyrifos blood levels at birth (672 hr/month / 104 hr half-life = 6.5 elimination half-lives per month). Also, the frequency and magnitude of pesticide applications would have a great impact on dose reconstructions of the Columbia study cohort. Unfortunately, this data was not measured in the Columbia epidemiology study (U.S. EPA 2014, p. 386-391). The investigators were also not able to estimate these data from the study questionnaires, as that data was deemed to be of low quality. Therefore, due to the lack of data on exposure scenarios and the fairly rapid clearance of chlorpyrifos, the method employed by Hattis (2013) of only calculating dose/blood level data from steady-state scenarios underestimates the variation in actual exposures in the Columbia study cohort. Accurate dose reconstruction would require additional data on temporality and magnitude of pesticide exposures, or alternately, simulations of a range of possible residential exposure timelines.

HHA Response: See response on page 36 of this document.

31. RCD Page 128-129:

VI.F.4. ToxCast and Zebrafish HTS Assays

DAS Response: A vast majority of the experimental studies that are cited as forming the basis for a proposed linkage between chlorpyrifos and effects on neurodevelopment and neurobehavior have methodological/design challenges which severely limit their utility in a weight-of-evidence assessment. These factors include dose, inappropriate route of exposure

(i.e., de-chorionation), utility of the neurotoxic vehicle DMSO, lack of solvent controls, inadequate replication, and a lack of analytical confirmation of the exposure concentrations. Collectively, these factors, along with other scientific perspectives and bases commented on earlier (see RCD Page 66 DAS Response) lead DAS to disagree with the DPR statement that "These studies provide strong weight-of-evidence for the ability of CPF to cause neurodevelopmental toxicity related to learning/cognition/behavior at doses 10x lower than those that cause AChE inhibition that would lead to neuromuscular effects in ZF (0.01 vs. 0.10 uM)."

HHA Response: Please see responses on pages 13 and 23 of this document.

32. RCD Page 130-131:

VI.G. Uncertainty Factors for Oral (Dietary and Non-Dietary) and Spray-Drift Risk Characterization

DAS Response: DAS has addressed DPR's comments/concerns regarding the PBPK modeling to develop DDEF values for pregnant workers with updates to the PBPK/PD model and recommends that the new modeling be considered and that a 4X UF is appropriate for all populations, including pregnant women. DPR also maintains that a 10X UF is needed based on "neurodevelopmental and neurobehavioral effects occurring in human fetuses in utero and during development…" Again, we remind the DPR that the epidemiology evidence is inadequate, inconsistent and biologically implausible. (Burns et al. 2013; Eaton et al. 2008; Li et al. 2012; Mink et al. 2012; Needham 2005; Prueitt et al. 2011; Reiss et al. 2015; Zhao et al. 2005) A vast majority of the experimental animal studies that are cited as forming the basis for a proposed linkage between chlorpyrifos and effects on neurodevelopment have methodological/design challenges which severely limit their utility in a weight-of-evidence assessment. These factors include dose, inappropriate route of exposure and utility of a neurotoxic vehicle, factors which have been highlighted by Scientific Advisory Panels (SAP's) as limiting the utility of reported results when considering relevance to humans. Several SAP reviews in addition to the EPA's Revised Human Health Risk Assessment (RHHRA) are consistent in their finding that a chlorpyrifos mode-of-action/adverse outcome pathway (MOA/AOP inclusive of non-cholinergic mechanisms) leading to neurobehavioral effects cannot be established. This has led to the EPA stating that "uncertainties such as the lack of an established MOA/AOP for neurodevelopmental effects and the potential exposure to multiple-AChE-inhibiting pesticides preclude definitive causal inference." Numerous independent reviews (including SAP reviews) have evaluated the body of data and purported studies/evidence that associate chlorpyrifos exposure with neurodevelopmental effects and there is consistency in the conclusion across these that protection against cholinesterase inhibition is protective of all other toxicities, including neurodevelopmental effects. Therefore, AChE inhibition remains the most robust quantitative dose response effect and thus continues to be the appropriate endpoint for use in quantitative risk assessment. Relative to risk assessment, there is no compelling or consistent animal or human data which would support retention of a 10X UF for concerns over neurodevelopmental/ neurobehavioral effects occurring at exposures below the threshold for RBC cholinesterase inhibition.

HHA Response: Please see our responses on pages 27, 31, 40, and 41 of this document. As mentioned previously, there have been several publications since the 2008 SAP which support the neurodevelopmental/ neurobehavioral effects from CPF. These studies have been included in the RCD and HHA scientists continually review new studies and findings as they are published. HHA will retain the 10x factor for uncertainties about the PBPK-PD model, especially as it relates to women of childbearing age.

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