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SUBJECT: Response to Selected Findings Submitted by the Office of Environmental Health Hazard Assessment on the DPR Draft Evaluation of Chlorpyrifos as a Toxic Air Contaminant (Risk Characterization Document dated December 11, 2017)

I. INTRODUCTION

On December 12, 2017, the California Department of Pesticide Regulation (DPR) received findings from the Office of Environmental Health Hazard (OEHHA) on their evaluation of the December 2017 Draft Evaluation of Chlorpyrifos as a Toxic Air Contaminant (TAC). The findings document titled "Findings on the Health Effects of Chlorpyrifos Relevant to Its Identification as a Toxic Air Contaminant" is available at http://www.cdpr.ca.gov/docs/whs/pdf/chlorpyrifos_oehha_findings.pdf. Several of the findings therein are similar to comments previously submitted by OEHHA to DPR on draft risk characterization documents on chlorpyrifos or are statements of agreement with DPR's conclusions in the draft TAC evaluation itself. As such, only OEHHA findings that are unique to the December 2017 draft TAC evaluation or that discuss new data or analyses are responded to below.

II. RESPONSES TO SELECTED OEHHA FINDINGS

OEHHA Finding #10. The respiratory effects of CPF may provide potential critical toxicity endpoints, and should be considered as such in the DPR analysis. Respiratory effects are the most commonly reported symptoms in bystanders in DPR's pesticide illness report (DPR, 2017b). There is additional evidence of CPF-induced respiratory effects in agricultural workers.

Among farmers in an epidemiological study evaluating the impact of pesticide exposure - the Agricultural Health Study - the OP insecticides (CPF, malathion, and parathion) were positively associated with wheeze; for the commercial applicators, the OP insecticides (CPF, dichlorvos, and phorate) were positively associated with wheeze (Hoppin et al., 2006). Exposure to CPF was strongly associated with wheeze in a dose-dependent manner in both groups.

Bystanders may be children, and the developing lungs of young children and those with respiratory problems can be more sensitive to CPF exposure due to various factors, including lung structure and limited detoxification capacity. The respiratory architecture of the developing lung is characterized by a much lower surface area compared with adults, resulting in an approximately 2-fold increase in respiratory tract exposure (per unit surface area) to particulates (Ginsberg et al., 2004; de Zwart et al., 2004; Sarangapani et al., 2003).

The metabolic capacity of the developing lung is also much lower than that of the adult. The majority of differentiation activity of pulmonary xenobiotic metabolizing enzyme systems occurs for an extended period of time after birth (Fanucchi, 2014). For example, CYP gene expression was found to be much greater in the adult versus fetal human lung (Choudhary et al., 2005). Lung carboxylesterase activity in neonatal (PND7) and juvenile (PND21) rats was estimated to be 27% and 64% that of the adult (PND90) (Karanth and Pope 2000). CPFOase (PON1 activity using CPFO as the substrate) in the neonatal (PND7) and juvenile (PND21) rat lung was about 8-fold and 1- to 1.8-fold lower than adult (PND90) levels, respectively (Karanth and Pope, 2000). These differences may lead to higher CPFO in the lungs of infants and children compared with adults.

> **HHA Response:** Hoppin et al. (2006) showed a dose related increase in the odds of wheeze episodes with increasing days of chlorpyrifos application. However, the authors do not indicate the exact amount of chlorpyrifos applied and, as such, quantitative assessment of the dose response cannot be performed with these data. The study by Hoppin et al. (2006), along with a series of papers on respiratory effects of chlorpyrifos including the newest 2017 Agricultural Health Study (AHS) results by the same investigators (Hoppin et al., 2017), will be presented in the final TAC document.
>
> Hoppin JA, et al. (2006). Pesticides and adult respiratory outcomes in the Agricultural Health Study. Ann NY Acad Sci 1076:343-354.
>
> Hoppin JA, et al. (2017). Pesticides are Associated with Allergic and Non-Allergic Wheeze among Male Farmers. Environ Health Perspect 125(4): 535-543.

OEHHA Finding #11. In the review of genotoxicity assays in the draft TAC document, CPF was found to be largely negative, with some positive effects found in yeast and bacteria. OEHHA notes that there are additional studies in the literature and should be considered in the overall evaluation of genotoxic potential of CPF, not only for oncogenicity concern, but also for other effects such as neurotoxicity (Muller et al., 2014).

HHA Response: It is well documented that DNA damage can disrupt proper functioning of the nervous system. DNA transcription is inhibited by single stranded DNA, adducts, and crosslinking, leading to neuronal cell death or slowly progressing neurodegeneration (Hetman et al, 2010). In fetal brain and in tissue culture, chlorpyrifos has been shown to alter expression of genes known to be involved in neurodevelopment and can induce changes in cell adhesion, cell migration, myelination, and long-term potentiation, as well as neuronal growth, axonal length, and neurite outgrowth (see Moriera, 2010 and Section VI.D.2.d. in the December 2017 draft TAC evaluation). However, none of these studies assessed if altered transcription or other effects on neuronal cells was due to DNA damage.

We reviewed the study cited by OEHHA (Muller et al, 2014) and will include a summary of the findings in the final TAC document. In this study, adult rats were treated subcutaneously with chlorpyrifos for 7 consecutive days. Neurotoxicity in the absence of AChE inhibition was evident at 0.1 mg/kg/day in two strains of rats. However, the atypical dosing route limited the utility of this study for establishing a critical NOEL. With respect to OEHHA's comment on the DNA damage-induced neurotoxicity, neurotoxicity occurred at about 100-fold lower doses (0.1 mg/kg/day) than genotoxicity (10 mg/kg/day) in the Muller study. In conclusion, chlorpyrifos may induce neurotoxicity via DNA damage at higher doses. However, we found no evidence of damage at lower doses concurrent with neurotoxicity.

Hetman M, et al. (2010). Neurotoxic mechanisms of DNA damage: focus on transcriptional inhibition. J Neurochem 114(6): 1537-1549.

Moreira EG, et al. (2010). Toxicogenomic profiling in maternal and fetal rodent brains following gestational exposure to chlorpyrifos. Toxicol Appl Pharmacol 245(3): 310-325.

OEHHA Finding #14. The point of departure (POD) is the starting point of a low-dose extrapolation and is used to determine the health risk associated with a certain exposure level. The PODs for all exposure routes and durations and sensitive populations were developed by DPR using a physiologically-based pharmacokinetic and pharmacodynamic (PBPK-PD) model. This model was developed by the registrant and used by US EPA for deriving the PODs for RBC AChE inhibition in its 2014 Human Health Risk Assessment (US EPA, 2014).

The PBPK-PD model estimated the air concentration or dose (dermal and oral) for 10% RBC AChE inhibition. For the residential bystander exposure scenarios, the PODs used to evaluate the risks are listed in Table 3. For inhalation, dermal, and incidental oral exposures, the steady state PODs were used in risk characterization. The use of the lower PODs in the draft TAC document, compared to the higher acute PODs, was said to compensate for background exposure to CPF. OEHHA finds that this is a conservative approach, but notes that it may add uncertainty to the risk estimate.

OEHHA notes that for inhalation exposure, the exposure expressed as air concentration is lower for children than females (13-49 years old). However, the exposures in terms of dose (mg/kg-day) are similar, when the DPR's default breathing rates are used for the conversion. On the other hand, the dermal POD for children is more than 5-fold higher than that for females (13-49 years old). An explanation for the biological basis for the differences in the magnitude of PODs would be helpful to support their use in the risk characterization.

> **HHA Response:** We examined the model parameters used to generate the PBPK-PD dermal PoDs for children 1-2 years old and females 13-49 years old in the 2014 US EPA revised risk assessment (Docket ID: EPA-HQ-OPP-2015-0653). The only difference in the modeling input files (m-files) for adults and children is the body weight. Children's body weight was set at 11 kg, which is 6.3-fold lower than the body weight for adults (69 kg). The dermal PoD for children (134.25 mg/kg/day) is 5.7-fold higher than that for females 13-49 yrs (23.60 mg/kg/day), which is about the same as the differences in their body weights.

OEHHA Finding #15. Overall PBPK-PD model application, construction and validation as well as the uncertainty and variability of the outputs are discussed in Findings 22 and 23 below.

There is uncertainty associated with the steady state inhalation PODs derived due in part to the difference in the physical characteristics of CPF between the inhalation model and the bystander exposure and the lack of model validation.

Inhalation exposure is the primary route of exposure from spray drift due to aerial, ground boom, and air blast applications, as noted below (Finding 16). In both the rat inhalation and human inhalation models, CPF was modeled as dry particles with relatively small sizes and assumed to be mostly (>90%) absorbed in the gastrointestinal tract following deposition in the respiratory tract and mucociliary clearance. The inhalation PK data of the PBPK-PD model were derived from an acute inhalation study in rats using dry particles in the respirable range (<10 μm) (Hotchkiss et al., 2010).

> **HHA Response:** Our analysis of available data shows that concentration rather than physical form determines the absorption and availability of chlorpyrifos (CPF) via inhalation route (Figure 1). As demonstrated in data compiled from three separate experimental animal studies (Hotchkiss et al., 2010, 2013 a, and 2013b), regardless of its physical form (i.e., CPF-oxon, CPF-vapor, or CPF-aerosol), the blood concentration of absorbed CPF expressed in terms of TCPy, oxon, and the parent compound increases with increasing CPF concentration. Hence, the assumption of 100% absorption employed in the exposure assessment and the PBPK model is appropriate for assessing the risk of chlorpyrifos via inhalation.

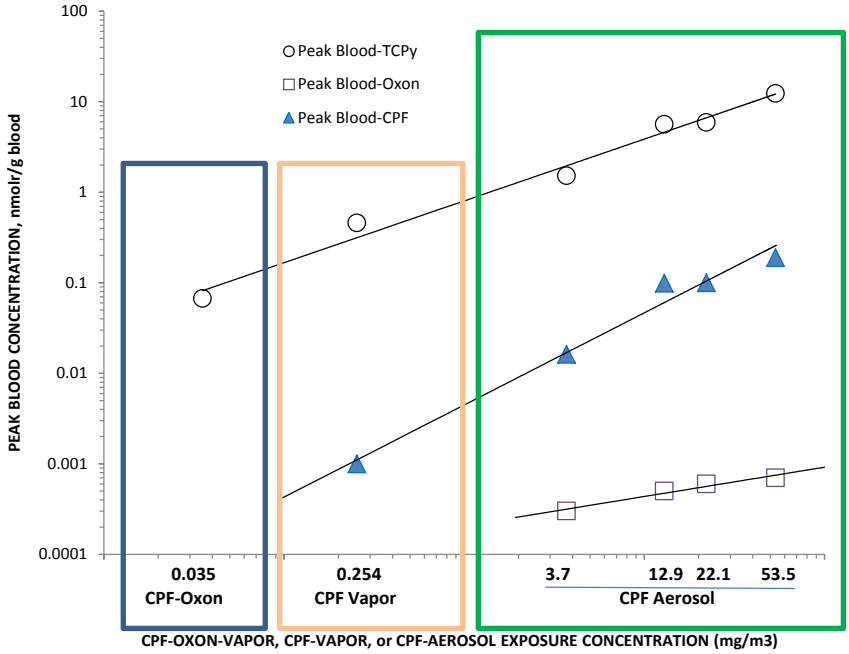

Figure 1. Peak Blood Concentration of TCPy, CPF-Oxon, and CPF from Inhaled CPF vapor, CPF aerosol, or CPF-Oxon in Rats (modified from Hotchkiss, 2010 and Poet et al., 2015)

Hotchkiss JA, et al. (2010). Acute Inhalation Exposure of Adult Crl:CD(SD) Rats to Particulate Chlorpyrifos Aerosols: Kinetics of Concentration dependent Cholinesterase (ChE) Inhibition In Red Blood Cells, Plasma, Brain, and Lung. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. MRID 48139303. (DPR Vol. No. 324-0908, Record No. 258214) 427.

Hotchkiss JA, et al. (2013a). Nose-Only Inhalation of Chlorpyrifos-Oxon Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity In Female CD(SD):Crl Rats. Dow AgroSciences LLC. (DPR Vol. No. 342-0950, Record No. 274123) 158.

Hotchkiss JA, et al. (2013b). Nose-Only Inhalation of Chlorpyrifos Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Female CD(SD): Crl Rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 4867 4: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. MRID 49119501. (DPR Vol. No. 324-0937, Record No. 271252) 178.

Poet TS, et al. (2015). Utility of PBPK/PD Model for Chlorpyrifos Exposures and Effects: In Depth Model description. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268.

OEHHA Finding #15, *continued*. In contrast, the bystander's inhalation exposure to CPF, as predicted by the AGricultural DISPersal (AGDISP™) model, is a spray drift cloud comprised of aerosol droplets of varying sizes that continually change as the cloud travels away from the application target. As larger droplets drop out, the cloud would have a greater portion of smaller droplets. The bystander at less than 25 feet from the application was estimated to be exposed to mostly "medium" and "coarse" spray droplets (Grisso et al., 2013). "Medium" and "coarse" are defined as droplets with diameters of 240 μm and 400 μm, respectively.

> **HHA Response:** The statement about bystander exposure in the OEHHA finding appears to be taken from Table 5 in Grisso et al. (2013), which shows the lateral movement in a 3-mph wind of a selection of discrete droplet diameters. Table 5 only summarizes a few discrete droplet sizes under a specific set of conditions for simple illustration (Grisso et al., 2013) and does not represent the behavior of the complete cloud of droplets released by the nozzles as simulated by the AGDISP model. For the RCD, the AGDISP simulations were performed under a 10 mph wind, which changes the lateral movement of droplets and the rate of droplet size change with time after release from the nozzles. The droplet spectra of the droplet cloud changes continuously as the droplet cloud travels downwind following release. The change in the droplet cloud size spectrum is complex and a function of wind speed, humidity, distance from the release, height above ground of interest (e.g., breathing zone of 5 ft.), and other variables. The AGDISP model accounts for the behavior of the entire droplet cloud as it travels downwind, which is why the model rather than a more simplified approach is used to estimate air concentrations. Appendix B of the December 2017 draft TAC evaluation (Barry, 2017) presents for a set of downwind distances the percent droplets in the droplet cloud that are below 10μm. Those results vary with aircraft and distance and are between about 3% of the droplet cloud at 10 ft to about 30% of the droplet cloud at 2608 ft. Even at 25 ft, approximately 4% to 10% of the droplet cloud is comprised of droplets 10μm in diameter or less.

OEHHA Finding #15, *continued*. Due to their large sizes, most of these droplets are expected to be deposited in the upper respiratory tract and absorbed in situ. Even if some of the smaller droplets reach the lower respiratory tract, they are likely to be absorbed in situ and not likely to be moved by the mucociliary mechanism and enter the gastrointestinal tract. In both cases, local effects of CPF on the upper and lower respiratory tracts would be a concern.

> **HHA Response:** The DPR analysis currently assumes that 100% of the droplet cloud is absorbed by the subject. Absorption is either through the gastrointestinal tract or through the lung.

OEHHA Finding #15, *continued*. Finally, the steady state outputs of the inhalation component of the PBPK-PD model have not been validated. There are no subchronic inhalation animal or human toxicity data suitable for this purpose. Although there are three subchronic inhalation toxicity studies conducted in rats (Newton, 1988; Corley et al., 1986; Landry et al., 1986), they cannot be used because the main reported effect was inhibition of plasma ChE; RBC and brain AChE were not inhibited.

HHA Response: We acknowledge that the available data are limited. However, the inhalation component of the PBPK-PD model has been validated using the human data derived from Vaccaro et al., 1993 and described in Poet et al., 2014.

Vaccaro J, et al. (1993). Estimation of the Absorbed Dose of Chlorpyrifos to Adult Volunteers following Treatment of Carpeting with Empire 20 Insecticide. Dow Chemical Co., Project # DECO-HEH2.1-1-182(123): HEH2.12-38-1(32).

Poet TS, et al. (2014). Chlorpyrifos PBPK/PD model for multiple routes of exposure. Xenobiotica 44(10): 868-881.

OEHHA Finding #16. Residential bystanders who are adjacent to a pesticide application are exposed to airborne CPF due to drift during or after the application. The draft TAC document assumed this was for 1 to 1.5 hours per day for 21 days. The scenarios evaluated in the draft TAC document are summarized in Table 4. The draft TAC document found that inhalation exposure contributed up to 95% of the total aggregate risk and contributions from exposures via diet and drinking water were minor. The spray drift and dietary aggregate exposure assessment was conducted only for children 1-2 years old, but not females (13-49 years old). While children often have the higher intake on a body weight basis, it is not clear from the draft TAC document whether the children group is the more sensitive group.

Table 4. Bystander exposure scenarios from spray drift of CPF.

Exposure Scenarios	Children 1-2 Years Old	Females 13-49 Years Old
Spray drift only Individual routes and all routes (Aggregate exposure)	Inhalation, dermal[a], incidental oral	Inhalation, dermal[a]
Spray drift and dietary aggregate exposure	All routes for spray drift plus CPF in food and to CPFO in the drinking water.	Not assessed

[a] Dermal- skin contact with airborne deposits on lawns or other outdoor surfaces.
[b] Incidental oral- transfer of residues from object (ie. a toy) to mouth, from hand to mouth, and from ingestion of soil.

HHA Response: The draft TAC evaluation did not assume or perform inhalation or dermal exposure for 1-1.5 hours every day for 21 days as suggested by OEHHA. The spray drift (inhalation or dermal) exposures were treated as short-term duration (1-1.5 hours). The 21 days exposure scenario was employed by US EPA for deriving route-specific PoD values in the agency's 2014 human health risk assessment of chlorpyrifos. (See HHA's Response to Comments Submitted by Dow AgroSciences, http://www.cdpr.ca.gov/docs/whs/pdf/chlorpyrifos_comments_dow_draft_eval_tac.pdf, page 4.)

OEHHA's additional concern is that spray drift and dietary aggregate exposure assessment was only conducted for children 1-2 years old, who may not be more sensitive than females 13-49 years old. We note that females 13-49 years old were

evaluated for dietary exposures from food and drinking water, as well for aggregate exposures from drift- dermal and inhalation routes. Both dietary exposure (Tables 53 and 55 in the December 11, 2017 draft TAC evaluation) and the drift exposure for females 13-49 years old (Table 43) were lower than those for children 1-2 years old. Overall, children 1-2 years old experienced higher aggregate exposure and risk.

OEHHA Finding #17. Three application methods were considered in the draft TAC document: aerial, ground boom, and air blast. A bystander can be exposed to CPF in air and after it has deposited on soil or vegetation surfaces.

a. For aerial applications, DPR used the AGDISP™ model for predicting downwind deposition of CPF residues. The model was also used to estimate one-hour time-weighted average (1-hour TWA) aerosol concentrations at specific downwind distances and receptor heights.

b. For ground boom and air blast applications, DPR used the AgDRIFT® model to predict downwind deposition of CPF residues. This model uses empirical data from a limited number of field trials to estimate droplet deposition. The AgDRIFT® model cannot predict aerosol concentrations in air. Instead, DPR applied "reasonable worst-case" inputs for AGDISP™ to generate air concentrations to predict aerosol concentrations from aerial application and used them as "surrogate" aerosol concentrations for ground boom and air blast applications in the evaluation of inhalation exposure (DPR, 2017a).

OEHHA agrees that use of the surrogate aerosol concentrations (62-101 μg/m3) are appropriate because they are similar to air monitoring data by the California Air Resources Board (60-81 μg/m3) when adjusted for distance from the field. These concentrations are likely conservative estimates for ground boom applications. However, they could be underestimates from air blast applications under some scenarios as more spray drift (higher concentration) may occur when little or no foliage is present.

HHA Response: In July 2011, US EPA identified inhalation exposures of concern associated with aerial, ground boom, and orchard airblast application in their analysis, "Evaluation of Potential Risks from Spray Drift" (EPA, 2012a). Based on the analysis reported in EPA (2012a), label changes were proposed and made according to the "Spray Drift Decision Document (059101)" (EPA, 2012b). These changes were done in part to increase protection for children and other bystanders, and to which registrants voluntarily complied by agreeing to lower application rates and implement other spray drift mitigation measures. As of December 2012, spray drift mitigation measures and use restrictions appear on all chlorpyrifos agricultural product labels (EPA 2014).

The inhalation exposures of concern for ground boom and orchard airblast presented in EPA (2012a) were calculated using surrogate air concentrations estimated using fixed wing scenario air concentrations. So, the use of fixed wing estimated air concentrations as surrogates for ground boom and orchard airblast is not without precedent, and is similar to the approach used by HHA.

As stated in the RCD, it is likely that the air concentrations estimated for the fixed-wing aircraft are as high or higher than those associated with either ground boom or orchard airblast because of the higher ground speed and the higher release height of the spray from aircraft. The use of the fixed-wing aircraft air concentrations are acceptable surrogates for dormant apple orchard air blast applications because the aerial application scenario used to produce those air concentrations was for 50 swaths (easily applied by aircraft in 1 hour), resulting in an equivalent application size of 206.6 acres (Barry, 2017). The total mass of chlorpyrifos released from a 6 lb/ac application rate in the aerial application surrogate scenario is 1236 lbs. An orchard airblast application is done at a much slower rate and will not cover the same number of acres in 1 hr. Therefore, much less mass will be released. HHA used an orchard airblast scenario application with 60 swaths and a resulting size of 21.2 acres. The orchard airblast application equipment travels at a ground speed of approximately 2.5 mph. Using that ground speed and the swath parameters shown in Table 3 of Barry (2017), the orchard airblast scenario application would take approximately 150 minutes without including the time for turns at the end of each row. So, the entire application could not be made in 1-hr. In addition, the total mass released in the dormant apple application rate scenario (6 lb/acre) is 127.2 lbs, approximately an order of magnitude smaller that the aerial application scenario. Therefore, the aerial application releases vastly more mass available to drift off-site in 1-hr estimation period of the air concentrations.

US EPA (2012a). Evaluation of the potential risks from spray drift and the impact of potential risk reduction measures. Chlorpyrifos, PC Code 059101, DP Bar code 399483 and 399485. Memorandum dated July 13, 2012. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Washington, D.C. 20460. https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0850-0105

US EPA (2012b). Spray Drift Mitigation Decision for Chlorpyrifos (059101) July 2012. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Washington, D.C. 20460. https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0850-0103

US EPA (2014). Chlorpyrifos: Updated Occupational and Residential Exposure Assessment for Registration Review. PC Code: 059101. DP BarCode: D424484. December 29, 2014. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Washington, D.C. 20460. https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0850-0196

OEHHA Finding #19. There is a potential residential bystander exposure to CPF vapor produced by the deposited CPF aerosols. CPF is considered to be semi-volatile and has a relatively low vapor pressure at 25°C. In some regions of California where CPF use is high, summer daytime temperatures routinely reach or exceed 100ºF and this could turn the deposited aerosol material to a source of CPF vapor. For bystanders close to the application site, the concentration of CPF vapor is likely to be much lower than that of CPF aerosol in the first hour

following application. However, compared to the exposure to aerosol, the exposure duration to the vapor can last many hours after the application has ended.

HHA Response: US EPA (2014a, 2014b) reviewed newly submitted toxicology studies submitted together with the revised analysis of the volatilization data based on public comments (Reiss et al., 2013). Based on those evaluations, US EPA concluded that "…volatilization of chlorpyrifos does not present a risk of ChE inhibition from inhalation of CPF vapor…" (US EPA, 2014a). In addition, in the volume entitled "Chlorpyrifos: Reevaluation of the potential risks from volatilization in consideration of chlorpyrifos parent and oxon inhalation toxicity studies," US EPA reevaluated risks due to volatilization exposure to CPF or CPF-oxon and concluded that based on new data, there are no human health risks of concern anticipated for volatilization exposure (US EPA, 2014b).

Reiss, R, et al. (2013). A review of EPA's "Chlorpyrifos: Preliminary evaluation of the potential risks from volatilization." Submitted by Exponent. 1800 Diagonal Road, Suite 500, Alexandria, VA 22314. Prepared for Dow AgroSciences LLC. 9330 Zionsville Rd. Indianapolis, IN 46268. https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0850-0171

US EPA (2014a). Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review. United States Environmental Protection Agency, Washington D.C.

US EPA (2014b). Chlorpyrifos: Reevaluation of the potential risks from volatilization in consideration of chlorpyrifos parent and oxon inhalation toxicity studies. EPA-HQ-OPP-2008-0850-0192.

OEHHA Finding #22. An interspecies UF of 3-fold should be applied because there are uncertainties in the output of the PBPK-PD model: not all model parameters were derived from human studies, differences between the nature and location of absorption of particles in the model and the residential bystanders, and the model has not been adequately validated for human steady state exposures.

HHA Response: Our view on the inter-species UF for chlorpyrifos was summarized in the response to OEHHA comments to the 2015 RCD dated August 15, 2017. (http://www.cdpr.ca.gov/docs/whs/pdf/chlorpyrifos_oehha_response.pdf). OEHHA's recommendation is primarily based on the premise that the PBPK-PD model is not entirely a "human model." The issues of particle absorption and study validation were addressed above (see responses to OEHHA Finding #15).

The PBPK model inputs noted to have the greatest impact on interspecies variation are absorption in the gut, binding to acetylcholinesterase, and metabolic bioactivation and clearance of chlorpyrifos (Poet et al., 2014, 2017). Many of the input parameters were derived from humans and, as such, the resulting output accounted for human specific physiology and metabolism. A notable example is the description of chlorpyrifos oxon

removal by carboxylesterases. The distribution of carboxylesterases in animals differs considerably from humans. In rats, plasma contains high levels of carboxylesterases, whereas in humans carboxylesterases are not found in the serum. The PBPK-PD model correctly accounts for the absence of carboxylesterases of human plasma (Li 2005, Eaton 2008). When there were no human specific values, parameters were extrapolated from animals. It is a common practice in PBPK modeling and in risk assessment in general to use animal parameters scaled to humans when human data are not available. Scaling by ¾ body weight in carcinogenicity is one example of animal to human dosimetric adjustment (OEHHA, 2011). In conclusion, our review of the model parameters could not justify an increase of the inter-species UF from 1 to 3 as suggested by OEHHA.

Poet TS, et al. (2014). Chlorpyrifos PBPK/PD model for multiple routes of exposure. Xenobiotica 44(10): 868-881.

Poet TS, et al. (2017). Use of a probabilistic PBPK/PD model to calculate Data Derived Extrapolation Factors for chlorpyrifos. Regul Toxicol Pharmacol. 2017 Jun;86:59-73.

OEHHA (2011). Notice of Amendment - Title 27, California Code of Regulations Amendment of Section 25703(a)(6): Quantitative Risk Assessment. http://www.oehha.ca.gov/prop65/law/111111notice.html

OEHHA Finding #23. An intraspecies UF of 30 is needed to fully account for the potential variability in both PK and PD in the human population. An UF of 10 is not sufficient as the PBPK-PD model did not fully account for physiological, anatomical, and biochemical changes during pregnancy and among different age groups. As DPR noted, sensitive parameters related to metabolic clearance of CPF and CPFO were based on data from a small number of plasma and liver postmortem tissues (Smith et al., 2011) (Table 6), and metabolic activities between live and preserved human microsomes may not be concordant.

Table 6. Number of *in vitro* samples used in deriving model input parameters, by age groups.

Tissues[a]	Infants < 1 year old	Children 1-2 years old	Children 3-17 years old	Adult ≥ 18 years
Plasma	10	1	6	3
Liver	8	5	8	9

[a] From Smith et al. (2011). Total of 20 plasma samples from 0.01 to 46 years old and total of 30 liver samples from 0.04 to 75 years old.

The draft TAC document described the derivation of Data Derived Extrapolation Factors (DDEF) for acute oral exposure in humans by Poet et al. (2017). The DDEF in this case was defined as the ratio between the oral doses for 10% RBC AChE inhibition for the median individual (50th percentile) and the sensitive individual (e.g., 1st percentile). The different percentiles were calculated by varying pharmacokinetic variables in the PBPK-PD model, as described below. Poet el al. found the calculated DDEFs are not large for different age groups: adult (3.4), infants (3.6), non-pregnant female (3.4), and pregnant female (2.9). These DDEF estimates are used to justify the intraspecies UF of 10 in the draft TAC document.

OEHHA is concerned about the small sample size in the raw data and the reliability of the method that was used to extend the variability range of the parameters. Using sensitivity analysis, Poet et al. determined that four key metabolic enzymes contributed over 80% of the variability in the model output. The four enzymes are CYP450 to TCPy, CYP450 to CPFO, hepatic PON1, and plasma PON1. Because there are very few age-specific *in vitro* tissue samples for these enzymes (Table 6), Poet et al. extended their ranges by using a boot strap method, assuming the four parameters are log-normally distributed, and conducting Monte Carlo simulations.

However, OEHHA notes that it is unlikely that the few samples of a given enzyme in Smith et al. (2011) can cover the full range of values within a given age group, especially at the tail ends of a distribution. For this reason, there are uncertainties in the mean and range estimated for the log-normal distributions. It is not clear that by extending the ranges of these four sensitive enzymes, to what extent was Poet et al. able to address the limitation of the dataset in Smith et al. (2011). In addition, there is a need to account for the variability in the PD aspect of the PBPK-PD model for RBC AChE inhibition. The reported coefficient of variation (CVs) for the parameters (i.e., inhibition rate, degradation rate, reactivation rate) describing RBC AChE inhibition are relatively small, between 0.14 and 0.36 (Poet et al., 2017). For example, the inhibition rate was derived from Dimitriadis and Syrmos (2011). While the sample size was large (n=306), it consisted of only adult male hazardous material team workers and support staff. It is unclear how representative these mean and CV values are for the general population.

RBC AChE activity varies with age, pregnancy, and even between healthy adults. Newborn infant RBC ChE activity was reported to be only half that of adult activity (Miyazono et al., 1999; Vlachos et al., 2010). Adult activity was only reached by 4 months to 1 year of age (Karlsen et al., 1981; Ecobichon and Stephens, 1973). Hematocrit was reported to decrease over pregnancy (Cunningham, 2010; Abduljalil et al., 2012), with a concomitant decrease in RBC AChE specific activity.

Thus, OEHHA believes the intraspecies UF of 10-fold should be at least 30-fold to capture the full range of PK and PD variability for RBC AChE inhibition in the population, especially when this endpoint is used as a surrogate for DNT (See Finding 24). Many factors can influence an individual's susceptibility to developmental neurotoxicants, potentially resulting in a large inter-individual variability (Bellinger, 2009). These factors include: maternal stress and low socioeconomic status, sex, coexposures to other neurotoxicants and health co-morbidities, and genetic polymorphisms (Cowell and Wright, 2017; Dipietro and Voegtline 2017; Bellinger, 2009; De Felice et al., 2015).

> **HHA Response:** Our response to this finding has been previously detailed in responses to OEHHA comments dated August 15, 2017 (http://www.cdpr.ca.gov/docs/whs/pdf/chlorpyrifos_oehha_response.pdf). Here we extend the response by including recent data from Poet et al., 2017, which shows that large variability in inputs to the PBPK-PD model does not translate into the same variation in the RBC ChE activity (i.e., model output). For example, input variation in metabolism (CYP450 and PON1 activities in intestine, liver, and plasma) with %

coefficient of variation (CV) ranging from 40 to 140 will translate into of 85% to 105% AChE activity change compared to control (100%). For biochemistry, input variation (total cholinesterase amount, degradation, inhibition, and reactivation rates) with CV% ranging from 55 to 120 will result in an AChE activity change of less than 1% compared to control (100%). This is consistent with the bootstrap model simulation of the four key metabolic enzymes varied from 58 to 98- fold (Table 2 in Poet et al, 2107), but produced less than a 4-fold change in RBC AChE inhibition (Table 3 in Poet et al, 2017). In conclusion, the DDEF should address OEHHA's concerns about the variation in age-related metabolism of chlorpyrifos. Note that in the 2017 draft TAC evaluation, HHA did not base the choice of intraspecies UF on the DDEFs calculated by Poet et al., 2017. Figure 5 and Tables 2 and 3 below are reproduced from Poet et al (2017).

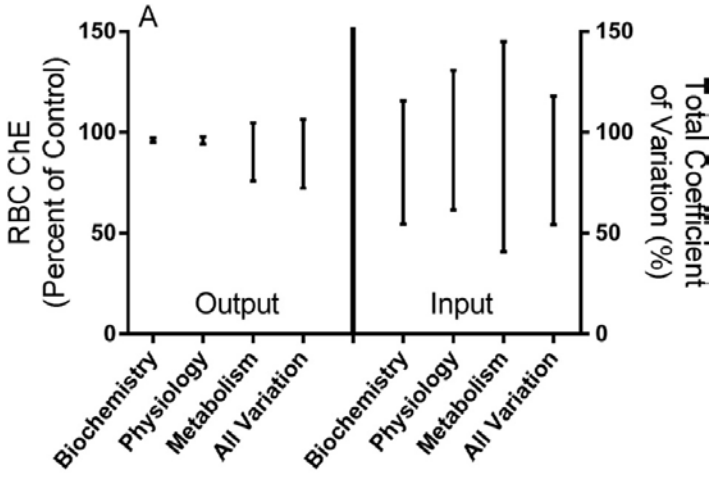

Fig. 5. A) Comparison of variability in model output and model input for different parameter distributions in a population of adult men and women. Lines show the total coefficient of variation normalized to the mean of the distribution for the output parameter (RBC cholinesterase) compared to the total CV for the input parameters for 3000 MC simulations (line). Physiology: body weight (tissue size), tissue blood flows, hematocrit, oral absorption rate; Biochemistry: total cholinesterase amount, and degradation, inhibition and reactivation rates. Metabolism, CYP450 and PON1 activities in intestine, liver, and plasma (See Table 1). B) RBC cholinesterase inhibition distribution for potential sensitive adult humans, showing the number of individuals exhibiting peak inhibition from 0 to 15%. There is a shift to the right (predicting more inhibition) when only PON1 activity is varied. Note-only up to 15% percent inhibition shown for clarity.

Table 2
Ratios of the maximum value to minimum value in the raw data, model output 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 in parametric distribution	26	34	33[a]	33[a]
Range in 20 parametric bootstraps	74	98	58[a]	58[a]

[a] PON1 in liver and plasma were assumed to be correlated and thus have the same variation.

Table 3
Data use in deriving the values of the Data Derived Extrapolation Factors for intra-species extrapolation ($DDEF_{HD}$).

	Adult male and female		Infants		Non-pregnant female		Pregnant female[a]	
	Median (50th percentile)	1st percentile	Median (50th percentile)	1st percentile	Median (50th percentile)	1st percentile	Median (50th percentile)	1st percentile
ED_{10} (mg/kg)	0.47	0.14	0.52	0.13	0.46	0.14	0.39	0.16
$DDEF_{HD}$		3.4		3.6		3.4		2.9

[a] Pregnant cohort is 3rd trimester, based on most sensitive group.

Poet TS, et al. (2017). Use of a probabilistic PBPK/PD model to calculate Data Derived Extrapolation Factors for chlorpyrifos. Regul Toxicol Pharmacol 86: 59-73.

OEHHA Finding #24. An additional factor is needed to address endpoints potentially more sensitive than RBC AChE inhibition. For DNT, the default UF is 10-fold; however, the use of this factor adds uncertainty to the risk characterization. There are several animal studies showing DNT effects at low doses (Table 2), and there are epidemiological data showing relationships between DNT and CPF exposure. OEHHA recommends a thorough evaluation of the studies to see if a POD for DNT can be directly determined.

HHA Response: In the December 11, 2017 draft TAC evaluation, HHA included summaries of the studies that identified DNT effects at doses lower than AChE inhibition. HHA will consider the suitability for deriving a PoD specific for DNT in the final TAC evaluation document.