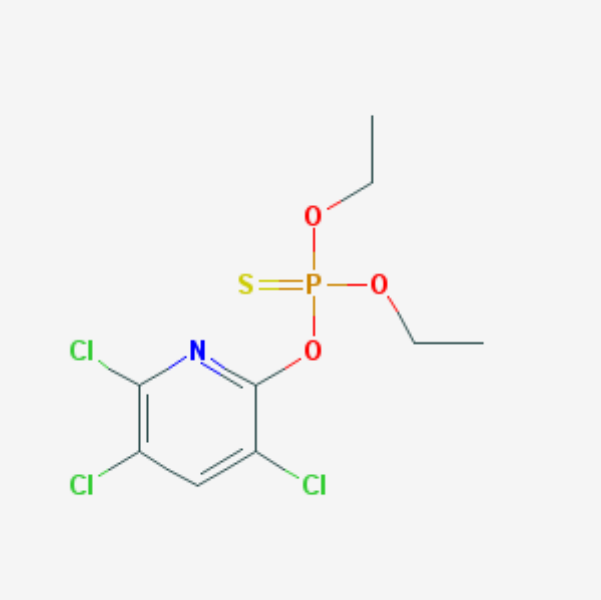
Final Toxic Air Contaminant Evaluation of Chlorpyrifos

Risk Characterization of Spray Drift, Dietary, and Aggregate Exposures to Residential Bystanders



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AADD	Annual average daily dose
AC	Adenylcyclase
AC_{50}	Active concentration resulting in activity of 50% of group
ACh	Acetylcholine
AChE	Acetylcholinesterase
ADD	Absorbed daily dose
AI	Active ingredient
BMD	Benchmark dose
BMDL	Benchmark dose lower limit (95^{th} percentile)
BuChE	Butyryl/plasma/pseudo-ChE or B-esterase
CalEPA	California Environmental Protection Agency
CCCEH	Columbia Center for Children's Environmental Health
CPF	Chlorpyrifos
CPF-oxon	Chlorpyrifos oxon
DAP	Dialkylphosphate
DPR	California Department of Pesticide Regulation
FIFRA	Federal Insecticide, Fungicide & Rodenticide Act
FQPA	Food Quality Protection Act
GABA	ɣ-aminobutyric acid
GD	Gestation day
GnRH	Gonadotrophin releasing hormone
HHA	Human Health Assessment Branch
HDT	Highest dose tested
IARC	International Agency for Research on Cancer
i.p.	Intraperitoneal
IRED	Interim Reregistration Eligibility Decision
IVIVE	In vitro to in vivo extrapolation
LADD	Lifetime average daily dose
LD	Lactation day
LDT	Lowest dose tested
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
LOD/LOQ	Limit of detection/limit of quantitation
MCL	Maximum contaminant level
MDL	Minimal detection limit
MOA	Mode of action
MOE	Margin of exposure
MTD	Maximum tolerated dose
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OP	Organophosphate

P450/CYP	Cytochrome P450s
PAD	Population adjusted dose
PBPK-PD	Physiologically-based pharmacokinetic-pharmacodynamic
PDP	Pesticide Data Program
PISP	Pesticide Illness Surveillance Program
PND	Postnatal day
PoD	Point of departure
PON1	Paraoxonase 1 or A-esterase
PPE	Personal protection equipment
ppm, ppb	Parts per million; parts per billion
PUR	Pesticide use report
RAS	Risk Assessment Section
RBC	Red blood cell
RED	Reregistration Eligibility Decision
RfC	Reference concentration
RfD	Reference dose
SADD	Seasonal absorbed daily dose
STADD	Short term absorbed daily dose
SAP	Scientific Advisory Panel
SRP	Scientific Review Panel
s.c.	Subcutaneous
SF	Safety factor
TAC	Toxic air contaminant
TCPy	3,5,6-trichloro-2-pyridinol
UF	Uncertainty factor
US EPA	US Environmental Protection Agency

EXECUTIVE SUMMARY

Chlorpyrifos is a chlorinated organophosphorus (OP) ester used as an insecticide, acaricide, and miticide. Chlorpyrifos has major uses in California as an insecticide for nut trees, fruit, vegetable, and grain crops as well as non-food crop uses (e.g., golf course turf, industrial sites, greenhouse and nursery production, sod farms, and wood products). Major use areas include the Central Valley, Central Coast region, and Imperial County. Use occurs year-round, with peak use during the summer. There are several dozen chlorpyrifos products registered by approximately 20 different companies. Methods of application allowed by labels include aerial, airblast, ground boom, chemigation, and others.

Chlorpyrifos first entered the comprehensive risk assessment process after being given a "High" priority status by the California Department of Pesticide Regulation (DPR) in 2011. Concerns originally focused on potential neurodevelopmental and neurobehavioral effects, genotoxicity and reproductive toxicity in rats, probable human exposure due to spray drift, possible hand-to-mouth exposure by children, and exposure through food and drinking water. The first draft risk assessment was published in December 2015. It was in that risk assessment that potential human exposure to spray drift (via inhalation or deposition) became a concern. As such, chlorpyrifos entered the formal evaluation process to determine the scientific evidence for listing it as a pesticide Toxic Air Contaminant (TAC) (CA Food & Agricultural Code §14021-14027).

Chlorpyrifos entered the formal TAC evaluation process and the first draft evaluation was published by DPR in August 2017. A subsequent revision was published in December 2017, which was reviewed by the Scientific Review Panel on Toxic Air Contaminants (SRP)[1]. This 2018 final TAC evaluation reflects the SRP's recommendation that DPR evaluate and identify the developmental neurotoxicity effects as the critical endpoint for the chlorpyrifos risk assessment.

This final TAC evaluation of chlorpyrifos reflects DPR's thorough evaluation of the developmental neurotoxicity effects as the critical endpoint for the chlorpyrifos risk assessment. Recent in vivo animal studies provide evidence of neurotoxicity to developing organisms at chlorpyrifos doses below those causing cholinesterase inhibition. Effects noted include altered cognition, motor control, and behavior in rats and mice. These studies, along with epidemiological studies, are the impetus for DPR considering developmental neurotoxicity as the

[1] With the enactment of California's Toxic Air Contaminant Act, the Legislature created the statutory framework for the evaluation and control of chemicals, including pesticides, as toxic air contaminants (TACs) (Food & Agricultural Code §14021-14027). The statute defines TACs as air pollutants that may cause or contribute to increases in serious illness or death, or that may pose a present or potential hazard to human health. DPR is responsible for evaluating pesticides as TACs. The law defines specific steps DPR must follow for the identification, evaluation, and control of pollutants in ambient air in communities across California. One of those responsibilities is to extensively evaluate the potential adverse health effects of candidate pesticide TACs and estimate levels of exposure associated with their use. The SRP must review the risk assessment to determine if it is seriously deficient based upon a review of the scientific data, the procedures and methods used to support the data, and conclusions.

critical endpoint for chlorpyrifos. As such, DPR's Human Health Assessment (HHA) Branch conducted a chlorpyrifos risk assessment using developmental neurotoxicity as the endpoint based on in vivo animal findings. A target MOE of 100 was selected to be protective of human health. The target is comprised of 10x for interspecies sensitivity, 10x for intraspecies variability, and 1x for potential neurodevelopmental effects. The resulting points of departure (PoDs), reference doses (RfDs), and reference concentrations (RfCs) are also shown in Executive Summary Table 1.

Protecting against Developmental Neurotoxicity

Identification of a rigorous neurodevelopmental point of departure for chlorpyrifos would be strengthened by elucidation of a potential mechanism. Mammalian neurodevelopment is multifactorial and there are likely multiple pathways involved, some of which may be mediated via the classical cholinesterase toxicity pathway of binding and inhibiting acetylcholinesterase (AChE). Other potential mechanisms maybe covariates of this pathway, or may involve other key events at the molecular, cellular, and tissue level. While an adverse outcome pathway has not been elucidated at this time, it is important to note that developmental changes have been documented in experimental animal studies at chlorpyrifos levels below those that inhibit AChE. There is also evidence of potential associations between in utero exposure to chlorpyrifos and altered human growth and behavior later in life in the epidemiological studies. There are acknowledged uncertainties in the human evidence, including a lack of exposure-effect relationships, inconsistencies in reported outcomes across studies, and quantitative measures of chlorpyrifos exposure that vary from study to study.

As such, DPR considered protecting vulnerable subpopulations from chlorpyrifos exposure and the potential neurodevelopmental effects through the use of developmental neurotoxicity and AChE inhibition endpoints, the latter which can be considered a surrogate for developmental neurotoxicity when adjusted by an additional uncertainty factor (UF) of 10, as described below.

1) **Point of departure based on neurodevelopmental effects.** Recent in vivo animal studies and human epidemiological studies have continued the investigations into the potential effects or associations of chlorpyrifos on neurodevelopment, growth, and behavior. HHA conducted a comprehensive review of recently available animal studies published from 2015 – 2018, especially focused on the potential for evidence of neurodevelopmental toxicity at low dose levels. Critical PoDs were established from animal studies reporting developmental neurotoxicity at dose levels that are generally considered lower than those necessary for AChE inhibition in red blood cells (RBC). As mentioned earlier, a target MOE of 100 was selected to be protective of human health. The target is comprised of 10x for interspecies sensitivity and 10x for intraspecies variability. There is no need for an additional UF for neurodevelopmental effects. The risk of exposures to inhalation and spray drift is exacerbated by consumption of food and drinking water in this approach.

2) **Uncertainty factor of 10x applied to an AChE inhibition endpoint to account for the developmental neurotoxicity.** In its December 2017 Draft TAC Evaluation, DPR added an additional UF of 10x to account for more sensitive neurodevelopmental effects than AChE inhibition, the critical endpoint used to characterize the risk from chlorpyrifos exposure in that draft evaluation. Effects on cognition, motor control and behavior have been reported in

the human epidemiology and in vivo animal toxicology studies, the latter occurring at doses 10-fold lower than the threshold established for RBC AChE inhibition. However, neither the human epidemiological studies nor the in vivo animal studies available for our review at the time of the December 2017 draft were sufficient to derive critical PoDs for neurodevelopmental effects. Adding an additional 10x UF (resulting in a total UF of 100 when combined with the UF of 10 for variation in human sensitivity) would account for the possibility of neurodevelopmental effects, thus increasing the protection factor of the estimated RfCs and RfDs for chlorpyrifos. By increasing the total UF to 300 (see Appendix 3), DPR has further increased the protection factor and the conservativeness inherent in the chlorpyrifos proposed target RfCs and RfDs. Based on the AChE inhibition endpoint, inhalation resulting from spray drift is the exposure risk of concern.

The description of the uncertainties associated with each of these endpoints and a discussion of the weight of evidence is found in the Risk Appraisal Section.

The developmental neurotoxicity database for chlorpyrifos is evolving and currently contains five in vivo animal studies that permit the establishment of a critical oral no observed effect level (NOEL). As will be demonstrated below, the dose at which the neurodevelopmental effects occurred in these studies were similar regardless of the exposure window or the duration of the exposure. The most important implication of the five studies is that the threshold for chlorpyrifos-induced neurodevelopmental effects following exposure in early life may be 10-fold lower than the reported threshold of 1 mg/kg/day established for RBC AChE inhibition.

This final evaluation, as with the previous drafts, is intended to evaluate chlorpyrifos as a pesticide TAC as defined in the California Code of Regulations, Title 3, Section 6864. The determination of a pesticide TAC is based on the air concentration, either measured or modeled, that exceeds the RfC divided by 10. As explained in the Risk Appraisal section and Table 29 later in this document, chlorpyrifos meets the criteria of TAC designation by using either the developmental neurotoxicity endpoint or the AChE inhibition endpoint, even without the additional 10x uncertainty factor necessary to account for the fact that the developmental neurotoxicity effects occur at a lower level than AChE inhibition (see the August 2017 draft TAC evaluation of chlorpyrifos, available at https://www.cdpr.ca.gov/docs/risk/rcd/chlorpyrifos_draft_evaluation_2017.pdf).

Executive Summary Table 1. Points of Departure and Reference Dose or Concentrations used to evaluate the Risk from Exposure to Chlorpyrifos in Selected Population Subgroups for Developmental Neurotoxicity

Route	PoD[a]	RfD[b] or RfC
Uncertainty Factors (UF)		**10 inter 10 intra 1 DNT**
Acute Oral [mg/kg/day] Infants Children 1-2 Children 6-12 Females 13-49	0.01	0.0001
Acute Dermal [mg/kg/day][c] Infants Children 1-2 Children 6-12 Females 13-49	0.104	0.001
Acute Inhalation [mg/m^3][c] Infants Children 1-2 Children 6-12 Females 13-49	 0.405 0.459 0.624 0.862	 0.004 0.005 0.006 0.009

[a] Point of Departure (PoD): The critical acute oral PoD for chlorpyrifos is a no-observed effect level (NOEL) for developmental neurotoxicity in animals based on changes in cognition, motor control and behavior in rats and mice (Lee et al, 2015, Silva et al, 2017, Carr et al, 2017, Gómez-Giménez, 2017, 2018).

[b] Reference Dose (RfD) or Reference Concentration (RfC): RfDs and RfCs are derived by dividing the appropriate PoD by the product of all uncertainty factors (UF).

[c] Route to route extrapolation:

Dermal: Route specific dermal PoD: oral PoD in animals (mg/kg/day) / dermal absorption in human (9.6% ; Thongsinthusak, 1991).

Inhalation: Route specific inhalation PoD: oral dose mg/kg/day / [Breathing Rate (BR) m^3/hr/Body Weight (BW) kg]; Oral PoD=0.01 mg/kg/day; Infants BR=0.188 m^3/h BW= 7.6 kg; Children 1-2 yrs BR=0.283 m^3/h BW=13 kg; Children 6-12 yrs BR= 0.417 m^3/h, BW=26 kg; Females 13-49 yrs BR=0.833 m^3/h, BW 71.8 kg (derived from Andrews and Patterson (2000) assuming 24-hr breathing rates of 0.59, 0.52, 0.38 and 0.28 m^3/kg/24 hr for infants, children 1-2 yr, children 6-12 yr and females 13-49 yr, respectively.) [See Appendix 4.]

I. INTRODUCTION

Chlorpyrifos is a chlorinated organophosphorus (OP) pesticide with a primary and well established toxicity pathway that involves the binding and inhibition of the enzyme acetylcholinesterase (AChE) by the oxon metabolite of chlorpyrifos. AChE hydrolyzes acetylcholine at synaptic clefts in the central and peripheral nervous systems and in some non-neuronal targets such as plasma and red blood cells. Exposure to high levels of chlorpyrifos may result in a cholinergic syndrome typified by respiratory distress, miosis, muscular twitches, tremors, ataxia, diarrhea, and vomiting.

Recent research has revealed that chlorpyrifos toxicity may extend beyond the classical cholinesterase-dependent pathway into more complex and often nuanced effects. Chlorpyrifos likely causes developmental neurotoxicity at exposure levels that do not induce overt toxicity in adult animals or inhibit cholinesterase activity. In contrast to the cholinesterase-based studies in animals and humans that were previously used to establish risk assessment endpoints, the five most recent studies show evidence of developmental neurotoxicity occurring at non-cholinesterase-inhibiting doses. Likewise, epidemiological findings provide likely evidence of an association between exposure to chlorpyrifos and impacts on growth and development. However, the measurement of specific biomarkers of exposure has been problematic in human studies, including major differences in analytical sensitivities and the common reliance on non-specific markers of exposure on which to base exposure-response relationships. Even with these challenges, there is a degree of concordance in the qualitative and quantitative effects seen in humans and recent animal studies, including changes in cognition, motor control, and behavior at low dose levels. Even so, deficiencies in quantified exposure analysis in epidemiological studies make it difficult to strictly compare those studies with the rodent DNT studies reviewed for this assessment.

History of Chlorpyrifos Risk Assessment in California

In its December 2015 draft risk assessment, DPR's Human Health Assessment (HHA) Branch initially adopted the points of departure (PoDs) from the 2014 US EPA Revised Human Health Risk Assessment for Chlorpyrifos (US EPA, 2014) which utilized an AChE inhibition endpoint. The PoDs were human estimates derived from physiologically based pharmacokinetic-pharmacodynamic (PBPK-PD) modeling of 10% AChE inhibition in red blood cells. It was in the December 2015 draft that the potential human exposure to spray drift (via inhalation or deposition) first became a concern. As such, chlorpyrifos entered the formal process to evaluate the scientific evidence for listing as a pesticide Toxic Air Contaminant (TAC) (CA Food & Agricultural Code §14021-14027).

The first draft TAC evaluation was published by DPR in August 2017. A subsequent revision was published in December 2017 which has been reviewed by the SRP. In the December 2017 Draft TAC Evaluation (see Appendix 6), the critical no-observed-effect level (NOEL) for evaluating oral, dermal, and inhalation exposure to chlorpyrifos was a PBPK-PD derived PoD based on 10% inhibition AChE after an acute (single day, 24 hr) or steady-state (21-day) exposure. The PBPK-PD model includes parameters that account for human-specific physiology and metabolism and can be used to derive age, exposure duration, and route specific PoDs.

Risks were calculated as a margin of exposure (MOE) for infants, children, youths, and non-pregnant adults. The MOE equals the critical PoD divided by the estimated human exposure level. DPR considered a MOE of 100 to be protective of human health for all exposure scenarios. The target of 100 included uncertainty factors (UF) of 1x for interspecies sensitivity, 10x for intraspecies variability, and 10x for potential neurodevelopmental effects. Exposures resulting in MOEs lower than the target of 100 are considered to be of potential health risk to humans.

Using the 10% AChE inhibition endpoint and exposures estimated from spray drift following aerial applications of chlorpyrifos, human health risks were identified from hand-to-mouth exposure to children, from inhalation exposure to children and women of childbearing age, and from various aggregate exposures. The air component of the exposure contributed up to 95% of the total aggregate exposure risk. Consequently, exposure to aerosols in the air near chlorpyrifos application sites was the main driver of the risk estimates of cholinesterase inhibition, especially for children 1-2 year olds, and thus substantiated the evaluation of chlorpyrifos as a TAC.

HHA revised its PBPK-PD modeling outputs for AChE inhibition as well as the resulting exposure estimates and MOEs (see Appendix 3). After further review of the PBPK-PD modeling parameters, and in consultation with the SRP, HHA subsequently increased the interspecies UF for model insufficiencies, thus adjusting the target MOE from 100 to 300.The revised PoDs, RfCs, and RfDs are found in Table 28 later in this document.

Also as part of their review of the December 2017 draft, the SRP recommended additional and detailed review of developmental neurotoxicity studies, in particular recent in vivo animal studies as well as a more in depth analysis of human effects of chlorpyrifos. In addition, the SRP recommended that DPR reevaluate the critical endpoints, the associated UFs, and the resulting RfCs and RfDs for each endpoint.

This final TAC evaluation of chlorpyrifos provides an update to the December 2017 draft and incorporates these changes.

II. TOXICOLOGY PROFILE

Recent in vivo animal studies and human epidemiological studies have continued the investigations into the potential effects or associations of chlorpyrifos on neurodevelopment, growth, and behavior. In finalizing this TAC evaluation, HHA conducted a comprehensive review of animal studies published from 2015 – 2018, especially focused on the potential for neuro-disruptive behavior at dose levels below those that cause overt cholinesterase inhibition. Care was taken to consider the timing of chlorpyrifos dosing, as well. Therefore in vivo studies are summarized by timing of exposure, e.g., gestation-only, postnatal-only, or combined dosing to provide comparison of results. The epidemiological studies reviewed herein are also new since the December 2017 Draft TAC Evaluation (Appendix 6). This section now also includes a review of new cohort and descriptive epidemiological studies as well as a comprehensive examination of the analytical methods used to quantify human exposure, which is important when considering the applicability of the epidemiological data to quantitative human health risk assessment. Also included in this revised Toxicology Profile is a review of a primate study and

discussion of potential mechanisms for DNT effects. This Toxicology Profile has been enhanced with a section on delayed neuropathy and neurodegenerative effects of organophosphate pesticides in animal, human, and mechanistic studies. Additional effects of chlorpyrifos have also been examined, including respiratory effects, glucose metabolism and obesity, and recent advances in PBPK modeling.

II.I. Developmental Neurotoxicity

The ability of chlorpyrifos to disrupt development is evaluated in this section. To this end, a series of studies was examined with the intent of establishing both a neurodevelopmental PoD and a plausible mode of action. A FIFRA-compliant developmental neurotoxicity (DNT) study submitted to fulfill registration data requirements under the California Birth Defect Prevention Act of 1984 (SB 950) was reviewed. This study evaluated the effects on neurological development following gavage exposure to chlorpyrifos in rats between gestation day 6 (GD 6) and postnatal day 11 (PND 11) (Hoberman, 1998). The study was originally summarized in the December 2017 Draft TAC Evaluation, however focusing on AChE inhibition. The updated review below provides a comprehensive review of all neurodevelopmental endpoints established in the Hoberman study. Furthermore, reviews of several in vivo animal studies published in the open literature from 2015 – 2018 have also been reviewed to provide as clear a picture as currently possible of the sensitivity of the developing nervous system to low doses of chlorpyrifos. Study findings and summaries are grouped according to the developmental periods in which the exposures occurred.

II.I.1. Gestational and Post-Natal Exposure to Chlorpyrifos

II.I.1.a. Hoberman (1998)

This registrant-submitted study examined the neurodevelopmental consequences of daily oral gavage exposure to chlorpyrifos in Crl:CD7(SD)BR VAF/Plus® pregnant rats (25/dose) during gestation and the perinatal period, GD 6 - PND 11 inclusive. Doses were 0 (corn oil), 0.3, 1, and 5 mg/kg/d. On GD 20, 5 dams/dose were sacrificed for measurement of plasma, RBC and brain ChE activities, in addition to examination of clinical, necropsy and reproductive parameters. On PND 5, 20 litters/dose were continued on treatment, from which four subsets consisting of 20 pups/sex/subset (1/sex/litter) were selected for evaluation of neurodevelopmental parameters as follows:

Subset 1: morphometric and histopathologic evaluations of brains after PND 12 sacrifice in 6/sex/dose;

Subset 2: Learning and memory evaluations by spatial delayed alternation (SDA) studies, including maze acclimation, acquisition training and delay training at PND 23-25 and 62-91in 8/sex/dose;

Subset 3: motor activity testing on postpartum days 14, 18, 22, and 61 (20/sex/dose) and acoustic startle response on PND 23 and 60 (20/sex/dose);

Subset 4: developmental landmarks (pinna unfolding, eye opening, preputial separation or vaginal opening) in 20/sex/dose; brain weight determination in 10/sex/dose sacrificed during PND 66-71, and neurohistopathology following *in situ* perfusion of 6/sex/dose.

Body weights were measured in all pups on PNDs 1 and 5 (pre-and postcull) and at several additional predetermined times (the latter for Subset 4 pups only). Positive non-concurrent controls were analyzed for neurohistopathology, spatial delayed alteration and motor activity, morphometry (PND 12 and PND 66-71) but not for acoustic startle response (Hoberman, 1999). Historical control brain morphometry data from the same laboratory but conducted after this study were available for 4-5 additional DNT studies (Hoberman, 1998). Finally, a satellite group consisting of 5 pregnant dams/dose was run to determine the effects of chlorpyrifos on maternal blood and brain cholinesterase on GD 20 (*i.e.*, after 2 weeks of exposure).

Maternal observations. Clinical signs in dams during the initial days of lactation included hyperpnea (1 mg/kg/d) and fasciculations, hyperactivity and hyperpnea (5 mg/kg/day). Neither maternal body weights nor food consumption was affected at any dose. Maternal plasma ChE was inhibited at the low dose on GD 20 (57% of controls; $p<0.0001$), with even greater levels of inhibition occurring at the mid and high doses. RBC ChE was also inhibited at the low dose (59% of controls, not statistically significant), with statistically significant inhibition occurring at the mid and high doses. Brain ChE was statistically inhibited at 1 mg/kg/day (18%; $p<0.0001$) and at 5 mg/kg/day (90%; $p<0.0001$) on GD 20. Benchmark dose analysis conducted by US EPA of the RBC ChE data generated BMD_{10} / $BMDL_{10}$ values of 0.06 / 0.03 mg/kg/day. US EPA analysis of the brain ChE data generated BMD_{10} / $BMDL_{10}$ values of 0.65 / 0.54 mg/kg/day (US EPA, 2011; p. 158). AChE inhibition by repeated doses of OPs, including chlorpyrifos, achieves a steady state degree of inhibition after 2 weeks of treatment. Similar levels of inhibition are observed after exposures of longer duration (subchronic or chronic scenarios). Thus the $BMDL_{10}$ for RBC and brain AChE inhibition from the current study were viewed by HHA as evidence of toxicity occurring after repeated exposures. In 2011, US EPA used the $BMDL_{10}$ of 0.03 mg/kg/day based on RBC AChE inhibition to characterize the risk from chronic exposure to chlorpyrifos (US EPA, 2011).

Pup observations. Neonatal pup losses, decreased pup growth, decreased pup body weight gains and developmental delays (represented by delayed pinna unfolding) were observed at 5 mg/kg/day. In addition, indicators of sexual maturation (preputial separation in males, vaginal patency in females) were delayed at that dose. The SDA maze studies conducted in PND 23-25 and 62-91 offspring did not yield convincing evidence for a chlorpyrifos-mediated effect. On the other hand, motor activity, gauged as the number of movements per 60-minute period, was reduced at 5 mg/kg/day in PND 14 pups compared to concurrent controls. No consistent pattern was present after that time (PNDs 18, 22 and 61). Measurements of peak acoustic startle response revealed possible reductions at 5 mg/kg/day in PND 23 and 60 animals. Similarly, the latency to peak response was greater in high dose animals on both days. Finally, two measures of sexual maturation, preputial separation in males and vaginal patency in females, showed delays at 5 mg/kg/day. All results are summarized in Tables 1 and 2.

Morphometric measurements for nine brain regions in PND 12 pups revealed statistically reduced cerebellar dimensions in high dose males (anterior-posterior decrease: 24.5%; height decrease: 14.2%; $p<0.05$) compared to concurrent controls (Table 3). As high dose male brain weights were 11.5% lower than concurrent controls, a chlorpyrifos -mediated impact on cerebellar growth in these males was considered to be possible. Other regions also exhibited

dimensional declines, but they were quantitatively less than, or similar to, the 11.5% brain weight decline, they couldn't necessarily be viewed as direct responses to chlorpyrifos.

Similar morphometric measurements were conducted in PND 66-71 adults, though the 0.3 and 1 mg/kg/day doses were omitted in males, as was the 0.3 mg/kg/day dose in females. The PND 66-71 measurements revealed statistically reduced parietal cortex dimensions in 1 and 5 mg/kg females (4% and 5%, respectively; $p<0.05$) (Table 4). Because control and 1 mg/kg/day female brain weights were unaffected, these changes were consistent with the possibility of a chlorpyrifos-mediated effect. In addition, non-statistically significant reductions in hippocampal gyrus dimensions in 1 and 5 mg/kg/day females (4% and 7%, respectively; $p>0.05$) may have resulted from chlorpyrifos exposure.

NOEL determinations in pups. A developmental lowest observed effect level (LOEL) of 1 mg/kg/day was established based on reduced parietal cortex and hippocampal dimensions in PND 66-71 female adults at 1 and 5 mg/kg/day. Morphometric observations were not made at 0.3 mg/kg/day; consequently, a discrete no-observed effect level (NOEL) could not be determined. In addition, cerebellar dimensions in PND 12 pups and hippocampal gyrus dimensions in PND 66-71 adults at 5 mg/kg/day were reduced. These observations were likely secondary to decreased pup growth over the course of gestation and perinatal development. Many other observations in pups, including body and brain weight decrements, neonatal pup losses, decreased pup growth, decreased pup body weight gains, decreased motor activity and developmental and sexual maturation delays, were observed at the high dose of 5 mg/kg/day.

NOEL determinations in pregnant dams. Because statistically significant inhibition of RBC cholinesterase was observed after 2 weeks of treatment in the pregnant dams at the low dose of 0.3 mg/kg/day, US EPA resorted to BMD analysis, generating a **maternal BMD_{10} / $BMDL_{10}$ of 0.06 / 0.03 mg/kg/day,** respectively. Brain cholinesterase underwent statistically significant inhibition at 1 mg/kg/day, generating BMD_{10} /$BMDL_{10}$ values of 0.65 and 0.54 mg/kg/day, respectively. These inhibitory effects were viewed as a result of repeated rather than acute toxicity. Clinical signs were noted at as low as 1 mg/kg/day.

Table 1. Effect of Daily Gavage with Chlorpyrifos in Pregnant Rats on Litter and Pup Parameters

	Dose (mg/kg/day) [c]			
	0	**0.3**	**1**	**5**
Surviving pups per litter				
Day 1	12.3	13.3	13.0	12.7
Day 5, pre-cull	12.2	13.1	12.7	8.9 [a]
Day 5, post-cull	10.0	10.0	10.0	8.7 [a]
Found dead (total pups / total litters)	1/25	2/24	2/24	50/23 [a]
Mean pup weight (g)				
Males: Day 1	6.6	6.7	6.4	6.1
Day 5, post-cull	9.8	10.2	10.1	8.8 [a]
Females: Day 1	6.3	6.2	6.1	5.6
Day 5, post-cull	9.4	9.6	9.5	8.2 [a]
Pinna unfolding				
% pups reached as of day: 2	7	3	1	0
3	48	47	47	17 [b]
4	94	99	91	71
5	100	100	100	99
Sexual maturation (day)				
Preputial separation, males	44.2±1.9	43.4±1.9	45.2±3.2	47±5.9
Vaginal patency, females	32.4±1.0	31.5±1.5	32.1±2.3	33.4±2.2*
No. of movements / 60 min				
PND 14				
Males	246±200	182±205	168±147	109±109
Females	228±197	238±208	183±207	145±126
PND 18				
Males	373±277	328±209	390±300	319±187
Females	343±268	402±234	357±226	520±239
PND 22				
Males	314±179	249±125	299±187	302±207
Females	229±88	258±174	253±153	347±207
PND 61				
Males	585±191	612±187	616±142	681±140
Females	635±164	693±97	701±144	743±102
Auditory startle habituation (g)				
PND 23				
Males	56.6±23.3	63.7±30.1	56.9±21.2	40.5±10.0
Females	59.9±18.1	57.6±16.0	55.7±17.4	48.7±20.5
PND 60				
Males	219.7±100.2	156.3±69.5	171.3±92.4	168.3±80.5
Females	146.6±81.2	145.5±89.2	97.0±47.6	133.7±82.3
Latency to peak auditory response (msec)				
PND 23				
Males	39.3±7.1	38.5±8.4	39.2±9.4	49.1±16.0
Females	37.1±8.8	36.8±7.0	38.2±7.0	43.0±7.5
PND 60				
Males	36.5±6.5	39.0±9.2	37.5±5.6	40.8±11.6
Females	39.3±9.2	43.4±9.4	45.6±11.3	43.1±8.8

* $p<0.01$

[a] Noted by the investigators as statistically significant.

[b] Noted by the investigators as not statistically significant. However, the apparent delay was consistent with body weight decrements and was thus considered to be treatment related.

[c] Values are expressed as arithmetic means ± standard deviations.

Table 2. Effect of Two Weeks of Daily Chlorpyrifos Gavage on Cholinesterase Activities in Pregnant Rats

Compartment	Dose (mg/kg/day) [a] 0	0.3	1	5
plasma (nmol/min/ml) (% of controls)	861.31±63.42 (100.00±7.36)	488.33±23.18*** (56.70±2.69)	268.15±35.04*** (31.13±4.07)	72.64±10.22*** (8.48±1.19)
RBC (nmol/min/ml) (% of controls)	652.50±235.34 (100.00±36.07)	363.31±105.03 (58.74±16.10)	101.72±44.35* (15.59±6.80	-0.88±0.98** (-0.13±0.15)
brain (nmol/min/g) (% of controls)	11296.28±315.01 (100.00±2.79)	11264.23±167.01 (99.72±1.48)	9274.83±316.47*** (82.11±2.80)	1149.97±104.14*** (10.18±0.92)

[a] Values are expressed as arithmetic means ± standard deviations.
*,**,***: p<0.05, 0.01, 0.0001, respectively, using one-way ANOVA

Table 3. Morphometric Observations in Postnatal Day (PND) 12 Pups after Daily Chlorpyrifos Gavage During and After Pregnancy

	Dose (mg/kg/d) 0	0.3	1	5	Historical controls (range)
Parameter [a]	**PND 12 males**				
Body weight (g) [b]	23.5±1.6	27.6±2.4 117%	25.9±2.4 110%	19.4±4.3* 83%	NA
Brain weight (g) [b]	1.28 ±0.04	1.41±0.07 110%	1.36±0.06 106%	1.17±0.16* 91%	1.24 (1.132-1.32) n=5
Brain / Bwt [b]	5.5±0.36	5.16±0.25 94%	5.3±0.36 96%	6.2±0.87 113%	NA
Cerebrum, ant.-post. (mm)	12.5±0.03	13.4±0.5 107%	13.1±0.49 105%	11.8±0.95 94%	12.2 (10.5-12.9) n=5
Cerebellum, ant.-post. (mm)	3.27±0.31	3.45±0.35 106%	3.33±0.19 102%	2.47±0.55* 76%	5.2 (3.2-6) n=5
Cerebellum, height (µm)	3504±129	3456±172 99%	3416±200 97%	3008±504* 86%	3410 (3005-3606) n=5
Frontal cortex (µm)	1348±53.5	1360±100.3 101%	1352±47.2 100%	1272±153 94%	1461 (1356-1551) n=5
Parietal cortex (µm)	1336±56	1448±58 108%	1448±32.8 108%	1256±138 94%	1483 (1409-1584) n=4
Caudate-putamen (µm)	2240±84	2240±108 100%	2312±93.2 103%	2224±148 99%	2400 (2304-2488) n=4
Corpus callosum (µm)	293±25.4	302±24.3 103%	290±35.7 99%	293±55.6 100%	285.7 (272-302) n=4
Hippocampal gyrus (µm)	904±93.2	1004±114 111%	972±54.2 108%	824±65.6 91%	1054 (948-1136) n=5
External germinal layer, cerebellar cortex (µm)	37.2±2	38.3±4 103%	40±7 108%	37.7±3 101%	35.9 (30.3-38.8) n=5
Parameter	**PND 12 females**				
Body weight (g) [b]	23.1±2.3	23.2±1.8 100%	23.1±2.8 100%	18.8±3.6* 81%	NA
Brain weight (g) [b]	1.28±0.08	1.28±0.04 100%	1.27±0.13 99%	1.17±0.13 91%	1.27 (1.08-1.34) n=5
Brain / Bwt [b]	5.59±0.37	5.53±0.36 99%	5.54±0.35 100%	6.36±0.87* 114%	NA
Cerebrum, ant.-post. (mm)	12.4±0.26	12.7±0.28 102%	12.8±0.63 103%	12.2±0.58 98%	12.2 (10.8-12.98) n=5

Cerebellum, ant.-post. (mm)	3.18±0.22	3.03±0.32 95%	3.3±0.17 104%	3±0.31 94%	5.09 (3.1-6) n=5
Cerebellum, height (µm)	3512±200	3176±130 90%	3120±328 89%	3208±226 91%	3404 (2856-3756) n=5
Frontal cortex (µm)	1376±92	1388±79.5 101%	1356±54.2 99%	1368±85.9 99%	1512 (1356-1616) n=4
Parietal cortex (µm)	1380±54.2	1376±19.6 100%	1368±80.3 99%	1304±72.3 94%	1513 (1423-1616) n=4
Caudate-putamen (µm)	2384±131	2224±116 93%	2288±108 96%	2152±134 90%	2398 (2328-2530) n=4
Corpus callosum (µm)	307±38.4	286±26.8 93%	304±35.7 99%	274±39.6 89%	281 (261-320) n=5
Hippocampal gyrus (µm)	936±81.7	912±50.3 97%	932±96.5 100%	828±78.5 88%	1014 (919-1060) n=4
External germinal layer, cerebellar ctx (µm)	38.7±3	36.3±6 94%	41.2±6 106%	40.8±6 105%	39.5 (36-44.8) n=4

[a] Values are expressed as arithmetic means ± standard deviations. Percentages refer to the percent of control values. Greyed boxes indicate brain regions for which morphometry was plausibly impacted by chlorpyrifos exposure.
[b] Body weights and brain body weight ratios were from Subset 1, PND 12 pups. Brain weight/body weight ratios were multiplied by 100.
* $p<0.05$; analysis conducted by the study investigators
NA = data not available

Table 4. Morphometric Observations in Postnatal Day (PND) 66-71 Adults after Daily Gavage with Chlorpyrifos in Pregnant & Postnatal Rats

	Dose (mg/kg/d)				**Historical controls (range)**
	0	**0.3**	**1**	**5**	
Parameter [a]	PND 66-71 males				
Body weight (g) [b]	388.9±24.9	385.4±35.6 99%	389.8±31.8 100%	348.0±31.8 89%	NA
Brain weight (g) [b]	2.30±0.069			2.30±0.021 100%	2.23 (2.127-2.4) n=5
Brain / Bwt [b]	0.59			0.66 112%	NA
Cerebrum, ant.-post. (mm)	15.9±0.400			16.18±0.264 102%	15.88 (14-16.7) n=5
Cerebellum, ant.-post. (mm)	5.7±0.232			5.67±0.216 99%	7.09 (6.27-7.6) n=5
Cerebellum, height (µm)	5152±218			5104±351.0 99%	5078 (4648-5419) n=5
Frontal cortex (µm)	1792±105			1768±75.4 99%	1791 (1660-1838) n=5
Parietal cortex (µm)	1756±79			1792±58.1 102%	1861 (1776-1956) n=4
Caudate-putamen (µm)	2800±176			2744±98.0 98%	3300 (2920-3624) n=4
Corpus callosum (µm)	266±29			247±17.9 93%	265.6 (243.2-285) n=4
Hippocampal gyrus (µm)	1640±92			1612±95.3 98%	1668 (1552-1819) n=5

Parameter [a]	PND 66-71 females				
Body weight (g) [b]	228.7±15.4	238.1±27.9 104%	228.8±20.6 100%	220.3±14 96%	NA
Brain weight (g) [b]	2.103±0.071		2.13±0.079 101%	2.05±0.05 97%	2.08 (1.93-2.15) n=5
Brain / Bwt [b]	0.92		0.93 101%	0.93 101%	NA
Cerebrum, ant.-post. (mm)	15.617±0.306		15.63±0.344 100%	15.52±0.248 99%	15.27(13.83-15.88) n=5
Cerebellum, ant.-post. (mm)	5.5±0.232		5.50±0.261 100%	5.38±0.098 98%	6.89 (5.77-7.38) n=5
Cerebellum, height (µm)	5016±120		4888±150 97%	4968±207.57 99%	4863.8(4592-5028) n=5
Frontal cortex (µm)	1744±56		1748±75 100%	1724±79.48 99%	1708 (1628-1818) n=4
Parietal cortex (µm)	1792±36		1716±36** 96%	1700±55.60** 95%	1738 (1656-1824) n=4
Caudate-putamen (µm)	2576±131		2552±178 99%	2704±112.23 105%	3142.8(2904-3379) n=4
Corpus callosum (µm)	244.8±25		258±18 105%	234±18.89 96%	264 (246-275) n=5
Hippocampal gyrus (µm)	1708±58		1644±149 96%	1592±86.76* 93%	1547 (1420-1602) n=5

[a] Values are expressed as arithmetic means ± standard deviations. Percentages refer to the percent of control values. Greyed boxes indicate brain regions for which morphometry was plausibly impacted by chlorpyrifos exposure.
[b] Body weights and brain body weight ratios were from Subset 4, postnatal day (PND) 66 pups. Brain weight to body weight ratios were multiplied by 100. The ratios in this table were calculated by DPR.
*,** p<0.05 & 0.01, respectively; analysis conducted by study investigators
NA = data not available; examination of external germinal layer of cerebellar cortex not completed in this group of animals

II.I.1.b. Gómez-Giménez et al. (2017)

Chlorpyrifos was dissolved in corn oil, mixed in a sweet jelly and fed to pregnant Wistar rats (6/dose). The females were treated from GD 7 to GD 20, then continued through lactation day (PND) 21 at doses 0, 0.1, 0.3 and 1.0 mg/kg/day. The purpose of the study was to determine (1) if spatial learning was affected in either sex after developmental exposure and (2) if hippocampal inflammation was associated with effects on spatial learning. There were no treatment-related effects on growth, number of offspring, survival, or bodyweights of the pups at any dose.

Cognitive Impairment Study: Pups were weaned on PND 21 and tested at age 2-3 months in the Morris water maze for effects on spatial learning.

1. Escape latency (Day 3) – pups were trained to learn the fixed location of a platform under water for escape (6-11 males/dose; 9 females/dose).
2. Reference errors (Day 4) – an 8-arm radial maze was used to record the number of first entries into an arm without pellets. In this test pups learn which 4 of the 8 arms have a food reward (3-10 males/dose; 9-10 females/dose).
3. Working memory (Day 5) – working errors were the number of entries into the 8-arm maze which the rat had entered previously (4-10 males; 9-10 females). A learning index was

calculated as the number of correct choices per number of errors for first entry into each arm of the radial maze.

Males were tested at all doses in all behavior tests, whereas female pups were only tested at 0.3 and 1.0 mg/kg/day. Escape latency in males increased at 0.1 mg/kg/day and above. Time spent in right quadrant on day 3 of testing was decreased in males at 1.0 mg/kg/day and unaffected in females. Spatial reference errors (first visits to unbaited arms) on testing day 4 were increased in males at ≥0.3 mg/kg/day. Working errors (visits to arms already visited in the same trial when seeking the baited arm) over the 5 days of testing increased in males at 0.3 mg/kg/day; females were not statistically significantly affected. Learning index (#correct choices ÷ #errors for first entry into each arm when seeking the baited arm) at day 4 decreased in males at ≥0.3 mg/kg. There was no apparent dose response in any of the effects. The authors conclude that chlorpyrifos impaired learning in males but not in females. **The LOEL for decreased spatial learning in males was 0.1 mg/kg/day.**

Inflammation Study: At 5-7 days after the behavioral tests were performed, rats (7-12 males/dose; 5-10 females/dose) were sacrificed and the hippocampus, a focal area for learning and memory, was dissected out to examine proteins that are markers of neuroinflammation (Iba-1, IL-4 and IL-10, IL-1b and TNF-α, GABA- α 1, GABA α 5 and GABA γ2, GluR1, GluR2, NR1, NR2A and NR2B). Protein assays were performed in males at all doses and at 0.3 and 1.0 mg/kg/d in females. Males exhibited decreased IL10 levels at 1.0 mg/kg/day in a dose-responsive manner that became significant at 1.0 mg/kg/day, while females showed decreases at 0.3 mg/kg/d and greater. IL-1b was increased at 0.1 mg/kg/day and greater in males but not in females. In contrast, Iba-1 was decreased in females at 1.0 mg/kg/d, while males were unaffected. The authors concluded that increased IL-1b in the hippocampus may correlate with the decreased spatial learning observed in males.

II.I.1.c. Gómez-Giménez et al., 2018

This study tested for potential gender-related effects of chlorpyrifos on spontaneous motor activity and motor coordination. Extracellular γ-aminobutyric acid (GABA) levels in the cerebellum and N-methyl-D-aspartate receptor (NMDR) subunit expression in the hippocampus were tested for possible associations. Extracellular cerebellar GABA modulates motor coordination (Chiu *et al.*, 2005; Hanchar *et al.*, 2005; Boix *et al.*, 2010); increased extracellular GABA has been associated with a decrease in motor coordination on the rotarod test (Boix *et al.*, 2010). NMDR subunit expression also affects motor activity and coordination. As in the previous study by this research group, pregnant Wistar rats were fed chlorpyrifos mixed in sweet jelly at 0 (n=10), 0.1 (n=4), 0.3 (n=4) and 1.0 (n=7) mg/kg/day, GD 7 through PND 21. The number of pups/dose (mg/kg/day) was 0 (22 males, 25 females), 0.1 (9 males, 5 females), 0.3 (18 males, 22 females), and 1.0 (21males, 20 females). The pups, weaned on PND 21, were tested at age 2-3 months for impacts on motor activity. Reproductive parameters were not affected in either sex.

Behavioral Effects: Spontaneous motor activity was measured in an open-field activity chamber (novel environment) using an actimeter (infrared motion detection). Motor coordination was measured by rotarod (constant minimum speed 2 min; increased from 4-40 rpm over 300 seconds). Females at 0.3 mg/kg/day exhibited decreased motor coordination on the rotarod.

There was a statistically significant increase in spontaneous motor activity in males and females at 0.1 mg/kg/day, but not at 0.3 or 1 mg/kg/day. **The LOEL was established at 0.1 mg/kg/d based on increased spontaneous motor activity in both sexes at that dose**.

Extracellular GABA and NMDR Levels: Assays for extracellular GABA in the cerebellum and NMDR subunit expression in the hippocampus were performed when the animals were 2-3 months of age. Microdialysis cannuli were implanted in the rat skull in half of the rats to allow access to the cerebellum in freely moving rats. Five samples of cerebrospinal fluid were collected for extracellular GABA analysis 3-7 days after performing motor activity tests. Brain tissue, dissected out and the hippocampus, was analyzed by Western Blot for NMDR subunit expression. Males exhibited no effects on motor coordination but showed increased extracellular GABA at 0.3 mg/kg/d (0.1 mg/kg/d not tested; dose responsiveness not apparent). There was no association in either sex between extracellular GABA subunits and motor coordination on the rotarod. However, males at 0.1 mg/kg/day who showed an increase in spontaneous motor activity also showed increased NMDA receptor subunits. On the other hand, females with increased spontaneous activity at 0.1 mg/kg/d showed decreased levels of NMDA receptor subunits. The NMDR pathway in the hippocampus is activated by glutamine and causes dopamine release in the nucleus accumbens, thus affecting voluntary motor activity (Peleg-Raibstein and Feldon, 2006; Barr *et al.*, 2014). However, a clear association in this study between spontaneous motor activity and NMDA receptor subunits was not detected in this study.

II.I.2. Gestational Only Exposure to Chlorpyrifos

II.I.2.a. Silva et al., 2017

Silva and colleagues investigated the effects on complex behaviors (particularly anxiety and depression) in Wistar rats exposed to chlorpyrifos in utero. Pregnant dams (11- 14/dose) received 7 consecutive daily doses of chlorpyrifos (0.01, 0.1, 1 and 10 mg/kg/day) by oral gavage on gestation days 14–20. Controls received the vehicle only (Tween 20 in 9% saline = 0.1 ml/ml). The last third of the gestation period was chosen because it is a critical period for fetal brain development and neurogenesis. Behavioral parameters in male pups were evaluated twice, during the infant-juvenile period (PND 21) and in adulthood (PND 70). Reproductive parameters (maternal body weight and weight gain, clinical signs of toxicity, gestation length, number of implants, post-implantation loss, mean pup weight, pup/dam ratios, number of live births and stillbirths, and male/female ratios at birth) were also examined. Male pups were separated into 4 groups (8-10 pups/group) comprised of those tested on PND 21 or PND 70. The elevated plus-maze test was used to assess anxiety levels. The open field test was used to evaluate locomotor activity. The modified forced swimming test was used to assess depressive behavior. Neither RBC nor brain AChE levels were measured, either in dams or in pups. Gestational exposures to 10 mg/kg/day chlorpyrifos resulted in reduced body weight gains in mothers during the treatment period. Maternal toxicity was not observed at lower doses. There were neither clinical signs nor effects on pregnancy that could be attributed to treatment.

Two tests conducted in PND 21 pups evidenced anxiety-like behaviors at maternal doses of 0.1

mg/kg/day and above. In the first test, time spent in the open arm of the elevated plus-maze was reduced by 45-50% at 0.1, 1 and 10 mg/kg/day ($p<0.05$).[2] And in the second, increased locomotor activity was detected in the open field test (30.3±3.43, 26.1±3.23, 40.6±3.28*, 52.1±5.26* and 42.3±5.66* intersections per 5-minute period at 0, 0.01, 0.1, 1 and 10 mg/kg/day; *$p<0.05$). The absence of a dose-related exacerbation of this response above 0.1 mg/kg/day was unexplained, but plausibly due to saturation of one or more of the many neural pathways involved in regulation of complex behaviors. There was no effect of chlorpyrifos on depressive-like behavior as evaluated in the modified forced swimming test. PND70 animals displayed neither anxiogenic (elevated plus-maze and open field locomotor activity test) nor depressive (modified forced swimming test) behaviors.

The authors concluded that chlorpyrifos treatment during pregnancy induced anxiogenic behavior in pups at the end of lactation (PND 21). As a result, they set the **LOEL for neurodevelopmental effects at 0.1 mg/kg/day. The lowest tested dose 0.01 mg/kg/day was the NOEL**.

II.I.3. Post-Natal Only Exposure to Chlorpyrifos

II.I.3.a. Mohammed et al. (2015); Buntyn et al. (2017); Carr et al. (2017)

Initial studies showed that male and female rat pups treated by oral gavage at 0 (corn oil) and 0.5 mg/kg/day during PND 10-16 exhibited behavioral anomalies when tested on PND 25. AChE was not measured. Decreased anxiety was evident through increases in number and percent of open arm entries, time and percent time spent in open arm of a plus maze, occurrences of crawling over/under, motor activity, play-fighting and time spent playing (Mohammed *et al*., 2015). In a subsequent study, pups were treated by gavage on PND 10-15 with 0, 0.5, 0.75 or 1 mg/kg/day chlorpyrifos (6-8/sex/dose) (Carr *et al.*, 2017). Forebrain AChE inhibition was noted at the high dose. Behavioral testing showed decreased times to emergence from a dark container into a novel environment at 0.5 mg/kg/day in both sexes. This behavior was associated with decreased anxiety. The data confirm earlier findings from this group showing that chlorpyrifos treatment generated behavioral effects at doses lower than those inhibiting brain AChE. The **LOEL for decreased anxiety in PND 25 pups was 0.5 mg/kg/day**.

II.I.3.b. Lee et al. (2015)

Male NMRI mice were treated by gavage with chlorpyrifos during rapid brain growth and maturation to investigate whether an acute perinatal exposure could be associated with behavioral effects in adulthood. Mammals undergo well-defined stages of neural development prior to full maturation, regulated by proteins (calcium/calmodulin-dependent kinases II (CaMKII), growth associated protein-43 (GAP-43), glutamate receptor 1 (GluR1), postsynaptic density protein-95 (PSD95), synaptophysin and tau control. These proteins are active during much of the brain growth spurt (BGS) stage (Wiedenmann and Franke, 1985; Navone *et al.*,

[2] Precise values are not provided for the elevated plus-maze test because the results were expressed in the form of histograms by the investigators.

1986; Benowitz and Routtenberg, 1997; Rongo and Kaplan, 1999; Ehrlich and Malinow, 2004; Wang and Liu, 2008; Traynelis *et al.*, 2010). The timing of the BGS in humans occurs from the 3rd trimester through age 2-3 years. In rodents the BGS occurs from birth through PND 21-28 (Semple *et al.*, 2013). The vehicle (20% fat emulsion/kg b.w. [1:10 egg lecithin + peanut oil]) used in this study was designed to simulate the fat content of mouse milk (~14%) in order to facilitate physiologically relevant absorption and distribution.

Treatment groups were as follows*:*

1. Brain AChE inhibition analysis: PND 10 pups received chlorpyrifos by gavage at 0 and 5.0 mg/kg (n=4/dose) as a single treatment. Assays were performed at 1, 3, 6, 12, 24 or 36 hours post-dose;
2. Neuroprotein analysis: PND 10 pups received a single gavage dose of chlorpyrifos at 0 and 5.0 mg/kg. These mice were sacrificed at 24 hours or 4 months after exposure and the hippocampus and cerebral cortex were frozen (n=5-8/dose)
3. Motor activity assessment: PND 10 pups were treated with chlorpyrifos by gavage at 0, 0.1, 1.0 and 5 mg/kg in a single dose followed by assessment at 2 or 4 months of age (n= 12/dose/time point). Locomotion, rearing and total activity were measured when mice were put in a novel cage and allowed to explore.

Results indicated 8-12% brain AChE was inhibition at 5.0 mg/kg (only dose tested: inhibition peaked at 3 h post-dose) which was reversed by 6 hours post-dose. CaMKII and synaptophysin were statistically significantly decreased by 42-50% at 5.0 mg/kg (only dose tested) 24 hours post-dose when brain AChE was no longer inhibited. The spontaneous motor behavior tests at 2 or 4 months after exposure showed statistically significant decreases in locomotion, rearing and total activity at 5.0 mg/kg. Total activity was statistically significantly increased at 0.1 and 1 mg/kg/day at 2 months and remained increased for the rats at 1 mg/kg/day at 4 months. The **LOEL for increased total activity was 0.1 mg/kg/day**, which is below doses causing brain or RBC AChE inhibition. The authors suggested that homeostatic disturbances during BGS of CaMKII may lead to irreversible behavioral effects lasting into adulthood.

II.I.4. Additional in vivo Animal Studies of Chlorpyrifos Reviewed

Reviews of two additional studies with chlorpyrifos in animals are included in this section: a long–term oral study with in non-human primates and a study with adult rats that were treated subcutaneously 7-day study (Coulston *et al.*, 1971; Muller *et al.*, 2014). Both studies showed that plasma ChE is more sensitive than plasma or RBC AChE. In addition, the rat study indicated that in adult neurotoxicity can occur in the absence of AChE inhibition. Neither of the studies established critical endpoints for repeated exposure to chlorpyrifos.

II.I.4.a. (Coulston et al., 1971)

Fourteen rhesus macaque monkeys (8 males and 6 females) were treated with chlorpyrifos by gavage for 6 months. The doses were 0, 0.08, 0.40, or 2.00 mg/kg/day (3-4 animals/group). Four males (1/group) were sacrificed at 3 months. Nine of the remaining 10 monkeys survived to sacrifice at 6 months. There were no effects on body weight, clinical signs, hematology, or clinical chemistry. Plasma ChE inhibition was observed at all dose levels starting from the first

measurement at 1 week, where 38%, 63% and 81% inhibition compared to pretreatment activities were noted at 0.08, 0.40, or 2.00 mg/kg/day (sexes combined), respectively, and continuing through week 24, where 34%, 70% and 62% inhibition were observed. RBC AChE was inhibited at the mid and high doses throughout the study. At 1 week, 0%, 11% and 67% inhibition were observed at increasing doses. At 24 weeks, 1%, 28% and 32% inhibition were observed. Two monkeys, a male and a female were evaluated for midbrain cholinesterase at 2.00 mg/kg/day. The male was sacrificed at 3 months and showed no difference from the control. The female was sacrificed at 6 months and had15% brain AChE inhibition. Midbrain AChE was not inhibited at the mid and low dose. The level of inhibition of brain AChE activity in the female after 6 months was comparable to values obtained for repeat-dose studies in the rat and dog. The NOEL was 0.08 mg/kg/day based on RBC AChE inhibition at 0.40 and 2 mg/kg/day after repeated treatment. The HHA Data Review Section classified this study as supplementary because it was not conducted according to FIFRA guidelines.

II.I.4.b. (Muller et al., 2014)

Investigators treated adult males rats (4-10/group) subcutaneously with chlorpyrifos at 0, 0.1, 1, or 10 mg/kg/day daily for 7 days. In Sprague-Dawley rats, the activities of plasma esterases, AChE, butylcholinesterase (BuChE), and carboxylesterase (CES) were measured, and comet assays and auditory startle tests were performed to assessed DNA damage and neurotoxic effects. Wistar rats received the same treatments prior to assessments of EEG's and somatosensory evoked potentials as measures of neurotoxicity. Inhibition of CES inhibition was significant at 10 mg/kg/day AChE $\geq$ 1 mg/kg/day and BuChE $\geq$ 0.1 mg/kg/day. The comet assay showed a significant damage index at 10 mg/kg/day. An assessment of startle response by a preceding sub-threshold sound pulse found significant attenuation at all dose levels, with nearly equal values for 0.1 and 1 mg/kg/day, and a marked reduction at 10 mg/kg/day. EEG recorded frequencies that were divided into 6 ranges and fractional power was calculated for each range. The 10 mg/kg/day group had more fractional power in the higher frequency ranges, which is consistent with an excitatory effect. For the somatosensory evoked potentials, rats were fitted with electrodes on the brain and the left paw. The paw was then stimulated and the evoked response was measured at the brain electrode. The response was recorded as positive peaks, negative peaks and latency. Negative peaks were significantly greater in magnitude than controls in all treated groups. The lack of apparent dose-response could be due to a saturable response at 0.1 mg/kg/day. Overall, a variety of parameters appeared to be affected at 10 mg/kg/day. 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.

II.I.5. Neurodevelopmental Mechanistic Studies

Identification of a rigorous neurodevelopmental point of departure for chlorpyrifos would be strengthened by elucidation of the potential mechanism(s). Mammalian neurodevelopment is multifactorial and there are likely multiple pathways involved, some of which may be mediated via the classical cholinesterase toxicity pathway of binding and inhibiting AChE or others that are covariates of this mechanism. While an adverse outcome pathway for chlorpyrifos-mediated DNT has not yet been elucidated, several recent studies have examined key events at the

molecular, cellular, and tissue level (reviewed (Burke *et al.*, 2017). These key events may involve other serine hydrolases such as monoacylglycerol lipase (MAGL) or fatty acid amide hydrolase (FAAH), oxidative stress, disruption of G protein-coupled receptors, changes in receptor tyrosine kinase (RTK) activity, disruption in ligand-gated ion channels, or chlorpyrifos-oxon mediated changes in neuronal growth (the latter reviewed in Eaton *et al.*, 2008). A full treatment of potential mechanisms for chlorpyrifos-mediated DNT and the proposal of an adverse outcome pathway are outside of the scope of this risk assessment. However, a review of current literature of chlorpyrifos related serine hydrolase disruption and disruption of adenylyl cyclase and serotonergic pathways can be found in Appendix 5 of this document.

II.K. Epidemiological Studies Related to Neurodevelopmental Effects

HHA completed a comprehensive search of human epidemiological studies that have investigated the correlation between exposure to pesticides and human development to more completely assess the available data beyond those published the December 2017 Draft TAC Evaluation. In addition, and at the suggestion of the SRP, HHA more closely reviewed the chlorpyrifos exposure analysis in these and other studies that were cited in previous drafts. Below is a summary of those findings and potential applicability of these results for quantitative risk assessment of chlorpyrifos.

II.K.8. Additional Epidemiological Studies

Several additional epidemiological studies have been reviewed. The cohorts or descriptive studies are generally focused on potential exposure to pesticide during pregnancy and consider study populations that reside in Bulacan, Philippines (Bielawski *et al.*, 2005; Corrion *et al.*, 2005; Ostrea *et al.*, 2006; Posecion *et al.*, 2006; Ostrea *et al.*, 2012), Central Ohio (Fluegge *et al.*, 2016), the Zhejiang Province, China (Wickerham *et al.*, 2012; Silver *et al.*, 2015; Silver *et al.*, 2017), and Mexico City, Mexico (Fortenberry *et al.*, 2014).

Bulacan, Philippines

A cohort study was initiated by Wayne State University and the University of the Philippines to consider fetal exposure to environmental toxicants. Pregnant women who resided in a rural area in the province of Bulacan, Philippines were enrolled at midgestation at the Provincial Hospital in Malolos. Over 598 mother/infant dyads and 638 individual infants were eventually recruited into the study. A preliminary survey of pesticides in home or farm use showed that 37% of study enrollees used chlorpyrifos (Ostrea et al., 2012). Maternal blood and hair samples were collected at midgestation and at birth, cord blood was collected at birth, and infant hair and meconium were collected within a few days after birth. Samples were analyzed for both parent pesticide and metabolites (Bielawski et al., 2005; Corrion et al., 2005; Ostrea et al., 2006; Posecion et al., 2006; Ostrea et al., 2012). Analysis of the meconium resulted in no detection of either chlorpyrifos or 3,5,6-trichloro-2-pyridinol (TCPy) (Bielawski et al., 2005). No maternal hair samples were positive for chlorpyrifos at midgestation and only 0.4% of the study population (n=2 of 449 subject) had detectable concentration of chlorpyrifos in hair at birth (median = 4.48 µg/g), which is slightly higher than the LOD of 4.15 µg/g. No maternal blood samples collected at midgestation or at birth were positive for chlorpyrifos (Ostrea et al., 2006) and no cord blood samples tested positive for chlorpyrifos (Ostrea et al., 2009). Additional samples were tested, and

only 1 of 282 mothers (0.35%) tested positive for chlorpyrifos in hair, with a concentration of 4.58 µg/g (Posecion et al., 2006). The investigators then analyzed the correlation between fetal exposure to pesticides and neurodevelopment as measured by the Griffiths Mental Development Scale at 2 years of age (95.1% follow up rate). Meconium was the most sensitive biomarker of fetal exposure to pesticides of all those analyzed (Ostrea et al., 2012). The Griffiths test evaluates 5 developmental parameters including motor skills, social acuity, hearing/language, eye and hand coordination, and visuospatial skills and reaction time. Because of the very minimal detection of chlorpyrifos or TCPy in any of the study samples, chlorpyrifos was excluded from further analysis (Ostrea et al., 2012). The only other birth cohort that analyzed meconium was the Columbia Center for Children's Environmental Health (CCCEH) study conducted at Columbia University, New York (Whyatt *et al.*, 2009). CCCEH researchers analyzed meconium for TCPy and not the parent chlorpyrifos, so it is difficult to compare results to the Bulacan cohort. It is interesting to note that in CCCEH meconium samples which had detectable TCPy above the LOD (0.2 ng; 28%), the highest concentration detected was 0.77 ng TCPy/g meconium (0.77 ppb) (Whyatt *et al.*, 2009).

Central Ohio

Fluegge et al. (2016) describe the effect of prenatal exposure to OPs as measured by maternal urinary metabolites and infant neurodevelopment ascertained at 3 months of age (Fluegge *et al.*, 2016). A cohort of 174 pregnant women were recruited from central Ohio from 2002 – 2005. Maternal urine was collected in the 2nd and 3rd trimesters, infant urine was collected at 2 months of age, and the neurodevelopment was assessed at 3 months using the Bayley Scales of Infant Development for 140 maternal-infant dyads. The arithmetic mean for maternal urinary TCPy (adjusted for body weight) was 26.69 (± 1.77) ng/kg/day, with a maximum measured of 334.72 ng/kg/day while the arithmetic mean for infant levels was 14.67 ng/kg/d (± 3.42) with a maximum measured of 399 ng/kg/d (Fluegge *et al.*, 2016). Third trimester maternal urinary TCPy was associated with impaired motor development ($p<0.05$) and infant urinary TCPy was associated with impaired mental development ($p<0.01$) both in the 3 month old infants (Fluegge *et al.*, 2016). Because TCPy is a metabolite of chlorpyrifos but also exists in the environment, it is difficult to ascertain how or if the mothers were exposed to chlorpyrifos parent compound, especially since measurements of the pesticide were not included in the study.

Zhejiang Province, China

Investigators considered the link between development and pesticide exposure in China, one of the world's leaders in pesticide use and production. Investigators conducted a pilot study (Wickerham *et al.*, 2012) and a full-scale cohort of pregnant women who were enrolled during the 36th week of gestation from Fuyang Maternal and Children's Hospital in the Zhejiang Province of China (Silver *et al.*, 2015; Silver *et al.*, 2017). In the pilot study, pesticides were analyzed in umbilical cord blood at delivery. Chlorpyrifos was measured in 27 of 116 samples above the LOD (>0.05 ng/ml), with the maximum concentration measure at 0.26 ng/ml (Wickerham *et al.*, 2012). No mean measurement was reported, although the 90th and 95th percentiles were reported as 0.17 ng/ml. These values were not associated with measured birth outcomes such as low birth weight (Wickerham *et al.*, 2012). In the full cohort study conducted from 2008 - 2011, investigators performed cord blood pesticide analysis on 336 infants samples. Chlorpyrifos was detected in 136 samples, with a maximum measured concentration of 11.40 ng/ml and the 75th percentile reported at 0.76 ng/ml (LOD = 0.675 ng/ml) (Silver *et al.*, 2015).

When the same infants were assessed for development using the Peabody Development Motor Scales-2nd Edition (PDMS-2), no significant associations were found between measured OP concentrations and PDMS outcomes at 6 weeks of age (Silver *et al.*, 2017). However, chlorpyrifos concentrations were associated with lower scores in all PDMS measurements of fine and gross motor skills at 9 months of age. When compared to unexposed infants, chlorpyrifos-exposed infants measured significant deficits in reflexes ($p = 0.04$), locomotion ($p = 0.02$), grasping ($p = 0.05$), and visual-motor integration ($p < 0.001$), respectively (Silver *et al.*, 2017). In the most recent study examined, the same cord blood measurements of chlorpyrifos were also significantly inversely associated with decreased head circumference in the infants (0.44 cm reduction; 95th CI 0.88, 0.1cm; $p = 0.02$) (Silver *et al.*, 2018).

Mexico City, Mexico

Fortenberry et al. (2014) investigated the relationship between in utero chlorpyrifos, chlorpyrifos-methyl, or TCPy exposure and attention-deficit hyperactivity disorder (ADHD) in school aged children in Mexico City using urinary TCPy as a biomarker of exposure. Women were enrolled in the prospective birth cohort called the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study during 1999 – 2005. Mother and child pairs were re-invited to examine childhood and adolescent neurodevelopmental characteristics when the children reached 6 – 11 years of age (Fortenberry *et al.*, 2014). Three psychometric assessments were used to assess ADHD related symptoms; the authors note the assessment tools used are for screening only, not diagnosis of ADHA. A total of 230 samples were analyzed for TCPy, 90% of which were above the LOD of 0.10 ng/ml. The geometric mean was 1.76 ng/ml (95th CI 1.55, 2.02) (Fortenberry *et al.*, 2014). When comparing the highest and lowest TCPy concentration tertiles, the authors noted suggestive (non-significant) associations between increased ADHD index in the highest TCPy tertile in boys ($p = 0.06$) as well as increased attention problems for the middle but not the highest TCPy concentration tertile in girls ($p = 0.08$). There were no statistically significant associations between any tertile of material TCPy concentration and ADHD observations in children (Fortenberry *et al.*, 2014).

II.K.9. Quantitative Analysis of Exposure

Human environmental epidemiology studies are being considered more in more in quantitative risk assessment, so much so that the US EPA Office of Pesticide Programs published the *Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides* in December 2016. In that guidance, US EPA states that quantitative biomonitoring is more advantageous than other exposure assessment methods, however there are several limitations including: 1) biological samples are generally only taken from a single point in time and may not accurately reflect longitudinal patterns, particularly if exposures are highly variable; 2) there can be degradation and metabolism of chemicals in both the environment and human body; 3) biomarkers of exposure may differ between individuals for reasons other than exposure level (differences in metabolism, presence of co-morbidities, etc.); and, 4) uncertainties inherent in the measurements, such as whether the biomarker is measuring exposure to the parent compound or environmental degradates (US EPA 2016). Both Burns and colleagues (2013) and LaKind and colleagues (2014) have noted challenges in accurately assessing quantitative exposure analysis in epidemiological studies. Burns notes that there must be careful attention to the type and specificity of exposure metrics and the validity of outcome measurement when evaluating the likelihood of establishing causality (Burns *et al.*, 2013). LaKind has noted that the

quality of exposure assessment is a major determinant of the overall quality of any environmental epidemiology study and has designed a tool to evaluate the quality of epidemiology studies that include biomonitoring. That tool outlines the important components for biomarker selection and measurement including the biological relevance (i.e., the biomarker in a specific matrix has accurate and precise quantitative relationship with external exposure, internal does, or target dose) as well as method sensitivity, biomarker stability, sample contamination, method requirements, and matrix adjustment (LaKind *et al.*, 2014).

As detailed in the December 2017 Draft TAC Evaluation (DPR, 2017), chlorpyrifos has several specific and non-specific markers of exposure. Most of the recent studies examined herein are finding value in quantifying the most specific biomarkers for chlorpyrifos, such as measured parent compound in blood, hair, and meconium and TCPy in blood and urine. Doing so adds weight to any possible association, more so than measuring nonspecific urinary biomarkers.

Even when these specific biomarkers have been measured in studies, there have been noticeable variations in the analytical methods, making comparison of results across studies difficult. The only way to unequivocally identify chlorpyrifos exposure is by measuring the intact pesticide in biological samples. Chlorpyrifos quantitation in blood can provide an estimation of the target site dose. Umbilical cord blood can provide some idea of recent in utero exposure, although large quantities (> 30 ml) are generally needed to perform the analysis using ultrasensitive analytical techniques (Barr and Angerer, 2006). Analysis is further complicated by the inherently low concentrations of chlorpyrifos present in the blood (~ng/L, ppt range) compared to levels of urinary metabolites (Barr *et al.*, 1999; Barr *et al.*, 2002). TCPy is a product of both the activation and detoxification pathways for chlorpyrifos, and therefore cannot be directly associated with toxicity. Urinary TCPy can also indicate exposures to CPF-oxon, CPF-methyl, and triclopyr (Barr and Angerer, 2006; Whyatt *et al.*, 2009). Environmental and dietary exposure to TCPy can also occur (Barr and Angerer, 2006; Whyatt *et al.*, 2009), complicating the use of TCPy as a biomarker of exposure. Fortenberry et al. (2014) also noted that while there was good trimester-to-trimester consistency of the urinary TCPy measurements in their study, there was significant within-woman variability across trimesters, which decreases the reliability of TCPy as a biomarker of exposure. In addition, when comparing chlorpyrifos levels in maternal or cord blood samples and TCPy levels in urine from the same subject, there was no association found (Whyatt *et al.*, 2009). Below is a description of the varying analytical techniques and sensitivities reported when chlorpyrifos as a parent compound was measured in biological samples in epidemiological studies, also summarized in Table 5.

II.K.9.a. Columbia Center for Children's Environmental Health (CCCEH)
The CCCEH study is described in the December 2017 Draft TAC Evaluation. Briefly, the cohort enrolled pregnant nonsmoking women residing in Washington Heights, Central Harlem, and the South Bronx, New York originally to investigate the effects of ambient and indoor pollutants on birth outcomes and development (Whyatt *et al.*, 2003). Samples of cord blood (n=211) were collected near delivery and maternal blood (n=199) was collected within 2 days postpartum and analyzed at the CDC using solid phase extraction and gas chromatography – mass spectrometry (GC-MS) as described in Barr et al., 2002. Standards were originally prepared with donor sera obtained from the American Red Cross, however the samples contained detectable background pesticide residues, and could not be used (Barr *et al.*, 2002). The investigators instead used water for QC standards, which is a very different matrix then the study samples being analyzed. For

method validation, a standard curve was created from 0.25 – 400 pg/µl (ppb) and chlorpyrifos recovery was approximately 20% (Barr et al., 2002). Chlorpyrifos in maternal serum ranged from ND – 35 pg/g (mean = 4.8 ± 5.5 pg/g) and ND – 63 pg/g in cord plasma samples (mean = 4.7 ± 6.5 pg/g) with a method LOD of 0.5 – 1 pg/g (ppt) (Whyatt *et al.*, 2003). It is important to note several issues with the analytical results. First, the standard curve was developed in the low to mid pg/µl (ppb) range while the chlorpyrifos concentrations detected in the samples fell several orders of magnitude below the calibration curve, in the pg/g or ppt range. In addition, the method documented a minimal recovery of chlorpyrifos in samples of approximately 20% (Barr *et al.*, 2002). Therefore, the low detection frequency and imprecision likely underestimated the true chlorpyrifos concentrations in the samples. Barr and colleagues noted the imprecision can be attributed to such things as deterioration of pesticides in frozen serum, the instability of pesticides in the heated GC injection port, and/or instability due to the reactive nature of pesticides; the imprecision was approximately double that of studies that had higher detection limits (Barr *et al.*, 2002). During the April 2016 US EPA Scientific Advisory Panel, Dr. Barr noted that the method was developed primarily to optimize pyrethroids detection, not chlorpyrifos. While the method was not developed for chlorpyrifos, the CCCEH principal investigators used this methodology when samples were sent to CDC for analysis (US EPA/SAP 2016). As such, HHA has reduced confidence in the CCCEH analytical findings, which, if used, may result in correlating of adverse developmental effects to exposures that are underestimated.

II.K.9.b. Saint Peter's University Hospital, New Brunswick, NJ

The New Brunswick prospective cohort is described in the 2017 December Draft TAC Evaluation. Briefly, pregnant women scheduled for C-sections were recruited from Saint Peter's University Hospital from 2003 – 2004 in a study to investigate pesticide exposure in maternal and fetal biological matrices (Barr *et al.*, 2010). Maternal samples were taken pre-operatively and cord blood samples were collected within 15 minutes of delivery and analyzed for chlorpyrifos using a solid phased extraction GC-MS methodology detailed in Barr et al., 2002. Chlorpyrifos was detected in n=138 (98.6%) of maternal samples (mean = 0.09 ng/g ± 0.87) and n=148 (62.8%) of newborn samples (mean= 0.55 ng/g ± 0.73). Assuming that the same analytical method was used in the CCCEH study without improvement, the same weaknesses in sample analytical findings can be assumed.

II.K.9.c. Johns Hopkins Hospital, Baltimore MD

The Johns Hopkins Baltimore Tracking Health Related to Environmental Exposures (THREE) Study was a cross-sectional study of fetal exposure to pesticide mixtures in babies born between 2004 and 2005 (Neta *et al.*, 2010). A total of 341 cord blood serum samples were collected and nonpersistent pesticides were tested using the GC-MS method detailed in Barr et al., 2002. Of a total of 185 samples, only 5 samples (3%) tested above the LOD of 21pg/ml, with the range equaling <LOD – 14 pg/ml (Neta *et al.*, 2010). Because of the low number of samples in which chlorpyrifos was detected, those samples and chlorpyrifos were excluded from any further analysis. Note that while the authors state they are using the analytical method used published in Barr et al., 2002, the study LOD was higher than reported in the original methodology (21 pg/g).

II.K.9.d. Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)

The CHAMACOS prospective cohort is described in the December 2017 Draft TAC Evaluation. Briefly, OPs were measured in maternal blood collected shortly before delivery and in cord

blood collected after delivery. Measurements were only made in those participants with sufficient blood volumes for the analysis at the CDC using the solid phase GC-MS method described in (Perez *et al.*, 2010). The LOQ reported herein was higher than that reported in CCCEH studies and the authors ascribe the difference to aging equipment and the inclusion of pyrethroids in the analysis which reduced the sensitivity to chlorpyrifos (Huen *et al.*, 2012). Even with the lower sensitivity (LOQ = 21 pg/ml), chlorpyrifos was detected in 70.5% of maternal samples and 87.5% of cord blood samples (Huen *et al.*, 2012). The detections ranged from ND – 1385 ng/ml for mothers and ND – 1726 ng/ml for newborns, however the two maximum values were considered outliers as they were more than 100-fold higher than the 95th percentile, and were removed from subsequent analysis (Huen *et al.*, 2012). The authors note that the median values detected in this study were below the LOQ for both maternal (0.006 pg/ml) and cord blood (0.004 pg/ml) samples (Huen *et al.*, 2012), thus decreasing their confidence in the values.

II.K.9.e. Bulacan, Philippines

As described above in Section II.K.8., pregnant women residing in the province of Bulacan, Philippines were enrolled at midgestation. Maternal blood was collected at midgestation and at birth and cord blood was collected at birth. Samples were analyzed using sold phase extraction techniques and GC-MS (Bielawski *et al.*, 2005; Corrion *et al.*, 2005). Calibration standards were prepared to encompass the entire calibration curve range, from 0.10 to 25 µg/ml. Internal QC standards were prepared using whole blood from subjects with no exposure from which 3 positive and 1 negative control were created for each of 3 concentrations. The mean chlorpyrifos recovery [[spiked control conc/expected conc]*100] was 137.5% with an LOD of < 0.10 µg/ml (ppm) (Bielawski *et al.*, 2005; Corrion *et al.*, 2005). The authors noted that the very high recovery for chlorpyrifos may have been due to errors in spiking volumes or evaporation that may have increased the concentration of the standards. No maternal blood samples collected at midgestation or at birth were positive for chlorpyrifos (Ostrea *et al.*, 2006) and no cord blood samples tested positive for chlorpyrifos (Ostrea *et al.*, 2009).

II.K.9.f. Zhejiang Province, China

As described above in Section II.K.8., investigators conducted a pilot study and a full-scale cohort of pregnant women who were enrolled during the 36th week of gestation from Fuyang Maternal and Children's Hospital in the Zhejiang Province of China. A 30 ml umbilical cord blood sample was collected which underwent solid phase extraction and isotope dilution GC-MS using fetal bovine serum as blanks and positive controls with a serial dilution of 0.01 – 50 µg/L. In the pilot study, chlorpyrifos was measured in 27 of 116 cord blood samples above the LOD (>0.05 ng/ml), with a maximum of 0.26 ng/ml (Wickerham *et al.*, 2012). In the full cohort, chlorpyrifos was detected in 136 samples cord blood samples, with a maximum of 11.40 ng/ml (LOD = 0.675 ng/ml) (Silver et al., 2015). Authors note that the 90th percentile chlorpyrifos concentration reported in the present study (3.85 ng/ml) was several orders of magnitude higher than the maximum concentrations reported in US studies (Silver *et al.*, 2015).

Table 5. Analytical Quantitation of Chlorpyrifos in Maternal or Cord Blood Samples

Study (reference)	No. samples	Samples > LOD or LOQ (%)	Median (Range)	LOD or LOQ	Notes on Methodology
CCCEH, New York (Whyatt et al., 2003)					
Maternal blood	199	148 (74%)	3.1 pg/ml (ND – 35 pg/ml)	0.5-1.1 g/ml	Method in Barr et al., 2002 CPF recovery 18-21% Standard curve = 21 – 400 pg/ul (ppb) Standards in water not plasma/serum
Cord blood	211	150 (71%)	2.6 pg/ml (ND – 63 pg/ml)		
Johns Hopkins THREE Study, Baltimore MD (Neta et al., 2010)					
Cord blood	185	3 (1.6%)	Median NR (< LOD – 14 pg/ml)	21 pg/ml	Method in Barr et al., 2002 The LOD reported is higher than originally validated in Barr et al., 2002
Saint Peter's University Hospital, New Brunswick, NJ (Barr et al., 2010)					
Maternal blood	140	138 (98.6%)	0.0007 ng/g (ND – 10.09 ng/g)	0.001 ng/g	Method in Barr et al., 2002
Cord blood	236	148 (62.8%)	0.0007 ng/g (ND – 1.84 ng/g)		
CHAMACOS Cohort, California (Huen et al., 2012)					
Maternal blood	234	42 (17.9%)	0.006 pg/ml (<LOQ – 400 pg/ml; 95th%-ile)	21 pg/ml	Method in Perez et al., 2010
Cord blood	256	29 (11.3%)	0.004 pg/ml (<LOQ – 1330 pg/ml; 95th%-ile)		
Zhejiang Province, China (Wickenham et al., 2012; Silver et al., 2015, 2017)					
Cord blood (pilot)	116	27 (23.3%)	NR (ND – 0.26 ng/ml)	0.05 ng/ml	Method modified from Perez et al., 2010
Cord blood (full cohort)	336	136 (40.5%)	NR (ND – 11.40 ng/ml)	0.675 ng/ml	
Bulacan, Philippines (Ostrea et al., 2006; 2009)					
Cord blood only	598	0 (0.0%)	0.0 µg/g	<0.10 µg/ml	Method in Corrion et al., 2005 and Bielawski et al., 2005

NR = not reported

II.M. Delayed Neuropathy and Neurodegenerative Effects of Chlorpyrifos

Delayed neuropathy and neurodegenerative effects were assessed further based on suggestions received during the January and March 2018 SRP hearings. The following new information outlines both specific human, in vivo animal and mechanistic studies that examined exposure to

OPs and associations with delayed neuropathy, Parkinson's Disease (PD), and Alzheimer's Disease (AD). Neurodegeneration in the form of organophosphate-induced delayed neuropathy (OPIDN), PD and AD have been reported after acute high-dose exposure to chlorpyrifos where significant brain AChE inhibition has occurred. In addition to AChE inhibition, high-dose chlorpyrifos appears to also result in misfolding of proteins, disruption of axonal transport, and mitochondrial dysfunction.

II.M.1. Human Studies of Delayed Neuropathy

II.M.1.a Human Case Reports of Delayed Neuropathy

Lotti et al. (1986b) evaluated a 42 yr old man who attempted suicide by ingesting approximately 300 mg/kg chlorpyrifos. After 3 weeks the cholinergic signs disappeared. On Day 30, RBC AChE, BuChE and lymphocyte neuropathy target esterase (NTE) were inhibited 50, 90 and 60%, respectively. On Day 40 he developed clinical signs consistent with organophosphate-induced delayed neuropathy (OPIDN). Other more recent cases of OPIDN have been reported in the open literature, all associated with acute high dose ingestion of chlorpyrifos from suicide attempts ((Nand *et al.*, 2007; Thivakaran *et al.*, 2012; Ostwal *et al.*, 2013; Mendes *et al.*, 2017; Yalbuzdag *et al.*, 2017).

II.M.1.b. Human Epidemiological Studies of Delayed Neuropathy

Ross and colleagues produced a fairly comprehensive meta-analysis of neurobehavioral problems in human adults following low level exposure in occupational settings (Ross *et al.*, 2013). In that systematic review, the authors pooled data from 14 studies and over 1600 participants and found significant associations between low level exposures to OPs and consistent (although at times, small in magnitude) changes in psychomotor speed, executive function, visual-spatial ability, and working memory (Ross *et al.*, 2013). The meta-analysis was not specific enough to detail if any of these effects were specifically related to chlorpyrifos exposure, although three of the base studies noted occupational exposure within their study populations to chlorpyrifos alone or in combination with other pesticides. Steenland et al., (2000) conducted a case-control study paring termite applicators who used chlorpyrifos with non-exposed maintenance workers and correctional officers. In comparison to the non-exposed controls, the chlorpyrifos-exposed cases did not differ significantly on the outcome of 40 subclinical tests, however, they did perform significantly worse on hand flexibility and body movements with closed eyes. The applicators also reported more qualitative symptoms including problems with memory, increased emotionality, increased fatigue, and loss of muscular strength. The outcomes were worse for those applicators who had reported an acute OP poisoning some time in their job history (Steenland *et al.*, 2000). In a more recent investigation of adolescent male pesticide applicators in the Menoufia Governorate, Egypt, researchers have assessed the potential for effects of low level cumulative chlorpyrifos exposure (Farahat *et al.*, 2011; Rohlman *et al.*, 2016; Callahan *et al.*, 2017; Ismail *et al.*, 2017a; Ismail *et al.*, 2017b). The investigators have considered the relationships between cholinesterase activity, neurobehavioral performance, and chlorpyrifos exposure across two application/growing seasons in groups of young male applicators and non-applicators and found that neurobehavioral deficits including motor function and speed were negatively impacted and cumulated over time and directly correlated with TCPy concentrations in urine as well as BuChE inhibition (Rohlman *et al.*, 2016;

Callahan *et al.*, 2017; Ismail *et al.*, 2017a; Ismail *et al.*, 2017b). While not a delayed neuropathic effect, it is important to note the potential for sustained effects after chlorpyrifos exposure has ceased.

II.M.2. Animal Studies of Delayed Neuropathy

Seven studies in hens were conducted to evaluate the risk for OPIDN (Table 6). Note that FIFRA guidelines require that the age of hens must be at least 8 months since younger hens are less sensitive. Hens are the animal model of choice since they are more sensitive. Positive controls usually tested at the same time using tri-ortho-cresyl phosphate (TOCP). Guidelines only require behavioral and histopathology examination of the brain, spinal cord and peripheral. Some of the non-guideline studies analyzed neuropathy target esterase (NTE) activity instead of performing histopathological examinations.

Lotti et al. (1986a) also measured in vitro the 50% inhibition concentration (I_{50}) values for chlorpyrifos oxon of AChE and NTE in hen brains, human brains, and human blood. The I_{50} values for AChE and NTE in hen brains were 0.006 and 0.15 µM, respectively. In human brains, the I_{50} values were 0.013 and 0.18 µM, for AChE and NTE, respectively. In human blood, the AChE and NTE I_{50} values were 0.007 and 0.11 µM, respectively. These I_{50} values indicate chlorpyrifos has less affinity for NTE than AChE, suggesting it is not neuropathic, but the observation of ataxia in the hens at 90 mg/kg indicate otherwise. Capodicasa et al. (1991) also calculated fixed time (20 min.) I_{50} values for CPF-oxon in hen and human brain homogenates at 6 and 13 nM for AChE, and at 150 and 180 nM for NTE, respectively. Richardson et al. (1993a) conducted kinetic experiments using two different approaches. The I_{50} for AChE and NTE calculated from their k_i were 2.24 and 239 nM, respectively. Using a fixed-time (20 min) pre-incubation method the I_{50}s were 2.16 and 206 nM, respectively. These I_{50} values were similar to those reported by Lotti et al. (1986b) and Capodicasa et al. (1991), with CPF-oxon being a more potent inhibitor of AChE than NTE suggesting that chlorpyrifos does not cause OPIDN. However, their study did not find any evidence of OPIDN. No evidence of OPIDN was seen in 2 subchronic studies in hens based on lack of ataxia and histopathological lesions in one study conducted by Barna-Loyd *et al.* (1986) and only transient staggering gait and low NTE inhibition (19%) in another study conducted by Richardson et al. (1993b).

Table 6. Hen Studies for Chlorpyrifos-Induced Delayed Neuropathy

No./dose age	Dosing Regimen	Antidotes	Findings	Ref.[a]
10/dose 17 months	Once, capsule 0, 50 or 100 mg/kg	Atropine prior to dosing	No evidence of OPIDN based on behavior and histopathology at 50 or 100 mg/kg, NTE not measured	1
No./dose NR age NR	Once, oral gavage 150 mg/kg	NR	Ataxia at Day 20, ↓NTE (>80%) on Days 4-5, no histopathology performed	2
5/dose age NR	Once, oral gavage 60, 90, 120 or 150 mg/kg (in glycerol)	Atropine and physostigmine before dosing, atropine & 2-PAM after	↓NTE (60%) at 60 mg/kg, ↓NTE (80%) and ataxia on Day 25 at 90 mg/kg, no histopathology performed	3
12/dose 18 months	Once, oral gavage 0, 75,150 or 300 mg/kg (in corn oil)	Atropine only as needed up to 54 hrs after	No evidence of OPIDN, ↓NTE (76%) on Day 4 at 300 mg/kg, no histopathology performed	4
5/dose 18 months	Once, oral gavage 150 mg/kg (in glycerol)	Atropine prior, atropine & 2-PAM after	Ataxia and gait disturbances by day 12; ↓AChE (88%) and ↓NTE (43%), ↓CI (69%) and ↓ATP (55%), no histopathology performed	5
10/dose 8-14 months	91 Days, oral gavage, corn oil, 0, 1, 5, or 10 mg/kg/day	None	No ataxia or histological evidence of OPIDN	6
15-18/dose, 18 months	20 days, oral gavage, corn oil, 0 or 10 mg/kg/day	None	Transient staggering gait, no histopathology performed, ↓brain AChE (58-70%); ↓NTE (~18%)	7

a. References: 1. Rowe et al. 1978; 2. Lotti et al. 1986a; 3. Capodicasa et al. 1991; 4. Richardson et al. 1993a; 5. Salama et al. 2014; 6. Barna-Lloyd et al. 1986;7. Richardson et al. 1993b.
Abbreviations: OPIDN = organophosphate-induced delayed neuropathy; NTE = neuropathy target esterase; 2-PAM = 2-pyridine aldoxime methyl chloride or pralidoxime; AChE = acetylcholinesterase; CI = Complex I; ATP = adenosine triphosphate.

Salama et al. (2014) suggested that the inhibition of Complex I rather than NTE was the cause of OPIDN based on their research. Complex I (also known as NADH dehydrogenase) is one of the enzymes in the respiratory chain in the mitochondria. In the brains of hens treated with chlorpyrifos at 150 mg/kg, NTE inhibition was only 45% while Complex I inhibition was approximately 70%. ATP levels around 55% below controls. Since the inhibition of Complex I was greater than the NTE inhibition, they proposed that the reduction in ATP levels was more likely due to Complex I inhibition than NTE inhibition. They pointed out that TOCP also caused a very strong inhibition of Complex I (~90%), although the NTE inhibition was greater (~95%).

II.M.3. Mechanistic Studies of Delayed Neuropathy

The first cases of OPIDN were with industrial OPs like TOCP that were not potent inhibitors of AChE, but were potent inhibitors of NTE. When Lotti et al. (1986b) reported a case of OPIDN in

man from ingestion of chlorpyrifos, OPIDN was thought to be related to inhibition of NTE whose function was not well understood. If an OP was a more potent inhibitor of NTE than AChE, it was considered potentially neuropathic. Even with these OPs, an NTE inhibition greater than 70% was thought to be necessary to produce OPIDN. At that time, the aging of the OP-inhibited NTE was considered essential for development of OPIDN. Aging involves the loss of the alkyl group of the phosphoryl residue attached to NTE leaving a negatively charged phosphorylated NTE. It was noted that neuropathic OPs reduced retrograde axonal transport and that NTE was located in the microsomes of neurons, so it was suggested that they may be important in axonal transport. Cytoskeleton proteins, such as microtubules, neurofilaments, and microfilaments, were also thought to be involved in the pathogenesis of OPIDN.

After several decades of research regarding the structure and function of NTE, it is now known that NTE is a serine hydrolase that is a member of the patatin-like phospholipase (PNPLA) subfamily and is sometimes referred to as PNPLA6 ((Richardson *et al.*, 2013). It resides in the membranes of the endoplasmic reticulum (ER) with the highest concentrations in neurons and lymphocytes. As a phospholipase, NTE is primarily responsible for hydrolyzing membrane-bound lysophospholipids, although it can also hydrolyze phospholipids. Lysophospholipids can disrupt membrane structure by acting as detergents (Wijeyesakere and Richardson, 2010). NTE is thought to maintain the lysophospholipids concentrations to 0.5-6% of the membrane by of weight. With NTE inhibition, there is a loss of homeostasis in the membrane resulting in lysophospholipid micelles which solubilize regions of the ER membrane. This can then lead to a loss of calcium homeostasis in the cell since the ER is the primary cellular store of calcium which can then lead to unregulated activation of calpains (calcium-dependent non-lysosomal cysteine proteases) resulting in the breakdown of the cytoskeleton and accumulation of calcium in the mitochondria. Increased calcium in the mitochondria can affect the permeability of mitochondria and eventually result in axonopathy through apoptosis. Another serine hydrolase referred to as phospholipase A2 (PLA2) is primarily responsible for hydrolyzing phospholipids to lysophospholipids. Since it is a serine hydrolase it can also be inhibited by OPs. Based on this new understanding of NTE's function it has been proposed that if the ratio of the I_{50}s for NTE to PLA2 is greater than one, it indicates that an OP is potentially neuropathic.

Some of the understanding of NTE's function was the result of research using *Nte*$^{-/-}$ and *Nte*$^{+/-}$ knockout mice (Winrow *et al.*, 2003). With these mice, these investigators determined that the *Nte* gene is highly expressed in the hippocampal neurons, the Purkinje cells of the cerebellum, the spinal cord, the Leydig cells of the testes and the developing lens. *Nte*$^{-/-}$ mice did not survive past embryonic day 8 indicating that NTE is critical for neurodevelopment. *Nte*$^{+/-}$ mice survived to birth with ~40% less NTE activity in their brain, but were hyperactive. The heterozygous knockout mice were also more sensitive to the potent NTE inhibitor, ethyl octylphosphono-fluoridate (EOPF), with higher mortality rates at 6 and 10 mg/kg. At 1 mg/kg of EOPF, wild type mice exhibited hyperactivity similar to that observed in the heterozygous knockout mice without EOPF. Based on this finding, the investigators concluded from this that aging of NTE was not critical in the development of OPIDN, but it is simply due to the sustained loss of NTE activity.

Additional research with conditional knockout mice (NTE-cKO) further elucidated the role of NTE in the nervous system (Akassoglou *et al.*, 2004). In NTE-cKO mice the NTE deletion does not occur until after embryonic day 11 so that these mice survive to birth. In these NTE-cKO mice, swelling of the neuronal cytoplasm, disruption and loss of the ER membranes, abnormal

reticular aggregates and vacuolation, were observed primarily in the large neurons of the hippocampus, thalamus and cerebellum. The lesions seen in the NTE-cKO mice were qualitatively similar to those seen in adult OP-dosed mice (Read *et al.*, 2009). In this study the investigators noted that the distal degeneration of the long spinal axons of the medulla oblongata preceded the swelling of neuronal bodies. They found that the phospholipid, phosphatidyl-choline (PtdCho), was elevated in the brains of both NTE-cKO mice and OP-dosed mice, although the increase in OP-dosed mice was transient. The axonal damage seen in the OP-dosed mice was limited to the longest spinal axons while the NTE-cKO mice had larger areas of axonal damage suggesting a linkage between the phospholipid homeostasis and axonal damage. The investigators concluded that the similar neuropathic lesions in the OP-dosed mice and the NTE-cKO mice suggest these lesions result from disruption of mature axons rather than abnormal neural development.

Other evidence supporting the role of NTE in the axonopathy associated with OPIDN comes from the identification of several NTE gene mutations associated with various forms of motor neuron disease (MND). Rainer et al. (2008) performed a DNA analysis on a consanguineous family of 10 subjects (3 affected) with Ashkenazi Jewish ancestry and a nonconsanguineous family of 5 subjects (2 affected) of northern European ancestry which exhibited progressive spastic paraplegia and distal muscle wasting which resembled OPIDN. Several mutations in the *Nte* gene were found. In the consanguineous family the affected individuals were homozygous for the NTE mutation c.3034A→G in NTE's catalytic domain. The two affected subjects in the nonconsanguineous family were heterozygotes for two mutations in NTE's catalytic region; one mutation (c.2669G→A) in NTE's catalytic domain and another involving an insertion (c.2946_2947insCAGC) causing frameshift and protein truncation (p.S982fs1019). Amyotrophic lateral sclerosis (ALS) is considered one form of MND. Ticozzi et al. (2010) sequenced the *PON* genes (*PON1, PON2 and PON3*) in subjects with either familial ALS (FALS) or sporadic ALS (SALS). From eight FALS and three SALS cases they found at least seven mutations in PON genes that were not in the controls. The incidence of *PON* gene mutations in the FALS subjects was about 2.5% after adjusting for cases with *SOD1*, *TARDBP* and *FUS* mutations. Based on the low incidence of these *PON* mutations among FALS cases, the authors concluded they were not the main cause of FALS, but they proposed that the loss of anti-oxidative capacity of the paraoxonases contributes to the development of ALS.

There are some investigators who think the mitochondrial dysfunction associated with OPIDN is independent of NTE inhibition. Masscotte et al. (2005) evaluated the activity of Complex I-IV in the human neuroblastoma cell line (SH-SY5Y) and in primary dorsal root ganglia (DRG) with exposure to phenyl saligenin phosphate (PSP) and mipafox (which are neuropathic OPs), paraoxon (which is a non-neuropathic OP) and phenylmethyl sulfonyl fluoride (PMSF) (which is a non-neuropathic NTE inhibitor). They did not test chlorpyrifos. They found that PSP and paraoxon were the most effective in inhibiting Complex I and IV in SH-SY5Y cells, although the inhibition was greater with PSP. PMSF only inhibited these enzymes at the highest concentration tested and mipafox didn't inhibit either even at the highest concentration. When rotenone (Complex I inhibitor) or sodium azide (Complex IV inhibitor) were added in addition to the OPs, no further inhibition was seen. Only PSP significantly inhibited Complex II and III activities. In DRG cells, only PSP and mipafox significantly reduced Complex I, III and IV. These investigators suggested that the ability of PSP to inhibit ATP production is unrelated to NTE

inhibition because PMSF at 1 μM should have caused greater than 90% inhibition of NTE (not measured) and yet was only a weak inhibitor of Complex 1 and IV. Masoud et al. (2009) reported reduction in mitochondrial respiratory enzyme activities, Complex I (20-55%), Complex II (30-45%) and Complex IV (15-40%) in rats after being administered the neuropathic OPs, monocrotophos (20 mg/kg oral) or dichlorvos (200 mg/kg s.c.). They also reported increased lipid peroxidation based on malondialdehyde (MDA) levels (10-20%) and decreased reduced glutathione levels (10-50%) in various brain regions. They proposed that oxidative stress lead to the inhibition of these mitochondrial respiratory enzymes.

II.M.4. Parkinson's Disease

Parkinsonism-like symptoms have been occasionally observed after acute OP poisoning. These symptoms occur at approximately the same time as the more common intermediate syndrome (IMS). The intermediate syndrome (IMS) was first reported following acute organophosphate (OP) poisoning in Sri Lanka (Karalliedde *et al.*, 2006). This syndrome occurs in only about 20% of acute OP poisonings. IMS differs from the cholinergic crisis in that muscarinic symptoms are not observed. IMS differs from OPIDN not only in terms of onset (earlier), but the paralysis associated with it is proximal while with OPIDN the paralysis is distal affecting the long axons, although there is some CNS involvement. The parkinsonism-like symptoms referred to as the extrapyramidal syndrome (EPS) has an onset about the same time as IMS are (Hsieh *et al.*, 2001; Detweiler, 2014; Panda *et al.*, 2014). The symptoms of IMS can be distinguished from EPS in that the symptoms from IMS are thought to be due to excess acetylcholine (ACh) at the nicotinic receptors and include paralysis of respiratory, neck, proximal limb muscle and cranial nerves. By contrast EPS is thought to be due to imbalance between cholinergic and dopaminergic neurons in basal ganglia and substantia nigra. The basal ganglia is more vulnerable to xenobiotics, metabolic abnormalities as well as to vascular insult because it is rich in mitochondria, vascular supply, neurotransmitters and chemical content compared with other areas of the brain. Hsieh *et al.* (2001) proposed there is a critical level of AChE in the basal ganglia that is necessary to regulate the dopaminergic system and this level may be lower than necessary for hydrolyzing acetylcholine. This may explain why some cases of EPS occurred in absence of cholinergic signs, although it is not clear if it occurred in absence of AChE inhibition. Both syndromes are considered transient syndromes, but there a couple reports of irreversible Parkinsonism after the acute OP poisoning (Goel *et al.*, 2006; Kwon and Kim, 2014).

There have been a couple reviews of the numerous epidemiology studies evaluating the association of Parkinson's disease (PD) with pesticide exposure. Brown et al. (2006) reviewed 38 case-controls epidemiological studies (13 in the United States, 11 in Europe, 5 in Asia, 2 in Australia, one in South America and another in Nigeria) and found 12 with significant positive associations in many studies with odds ratios (ORs) ranging from 1.6-7.0. They noted associations were strongest for exposure to herbicides and insecticides and with long durations of exposure to pesticides. They also noted that the toxicological evidence was strongest for rotenone and paraquat specifically. Freire and Koifman (2012) reviewed various types of epidemiological studies evaluating pesticide exposure and PD. This included one cross-sectional study, 8 prospective studies, and 38 case-control studies. The cross-section study found an OR of 3.7 (95% CI 1.6 – 8.6) among Italian men with pesticide use licenses compared to those without a license. Among the 8 prospective studies, most reported positive associations with occupational exposure to pesticides with risk estimates greater than 2, except one recent study with Swedish

male twins which found no association. Of the 38 case-control studies, 23 only examined overall exposure to pesticides and PD risk. Thirteen of these 23 studies found significant ORs between 1.1 and 2.4. They noted that when specific pesticides were examined, insecticides were the most widely studied. Among insecticide groups, positive associations were found with organophosphates, organochlorines, arsenic and rotenone. Among herbicides, positive associations were found primarily with paraquat.

Chuang et al. (2017) examined the association of PD with OP or carbamate (CM) poisoning in a retrospective study involving a cohort of 45,594 patients (9,128 patients with a history of OP or CM poisoning and 36,466 control patients) that were part of the Taiwan National Health Insurance Research Database. The incidence rate ratio (IRR) for PD in OP or CM poisoned patients was 1.36 (95% CI 1.26 – 1.47). The incidence of PD in patients over 75 years old was 77.4% in patients with OP or CM poisoning, but only 43.7% in control patients. The age-specific relative risk was highest in those less than 50 years old (adjusted IRR = 3.88, 95% CI 3.44 – 4.39). They did not look at PD risk with poisoning by specific OPs or CMs or even separate risk analysis for OPs and CMs.

II.M.4.a. Human Epidemiological Studies of Parkinson's Disease

The Parkinson's Environment and Gene (PEG) project conducted a number of population based case-control studies in three rural central California counties (Kern Tulare and Fresno) in which they estimated ambient residential and workplace pesticide exposure using the DPR California Pesticide Use Reporting (PUR) data from 1974 to 1999 and GIS-based modeling. Use of PUR data and home and work addresses to estimate pesticide exposure avoids some of problems other case-control studies have due with recall bias and exposure misclassification from broad ever/never exposure categories. However, it should be noted that the PUR database before 1990 is not very accurate since full use reporting was not required at that time (http://www.cdpr.ca.gov/docs/pur/purmain.htm).

In one PEG study conducted by Gatto et al. (2009), PUR data was used to estimate well water pesticide exposure assuming that if that pesticide was applied nearby and was a potential groundwater contaminant and well water was their primary source of drinking water, then there was exposure to these pesticides in well-water (Table 7). Six pesticides that were water soluble were considered separately, including chlorpyrifos, diazinon, propargite, paraquat, dimethoate and methomyl. They considered people who did not use well water as their primary source of drinking water had ambient only exposure. Consequently, all exposures were theoretical since there was no environmental monitoring of well-water or air. It also does not appear that they factored in possible occupational exposure or household use of pesticides. This study included 368 PD cases and 341 controls that were mostly male (cases = 56.2%, controls = 51.6%) and predominately white (cases = 85.3, controls = 85.6%). The adjusted odds ratio (OR) for chlorpyrifos was 1.87 in the high exposure group (95% CI 1.05 – 3.31). The authors also note that well water could also be contaminated with multiple agricultural and industrial chemicals as well as metals.

In another PEG study, the authors looked at the incidence of PD among different genotypes of PON1 (Manthripragada *et al.*, 2010). The PD cases (351) were mostly male (57.4%) and predominately white (80.4%) compared to controls (363) which had fewer males (46%) and

whites (69.9%).Among the PD cases the frequency of this *PON1*$_{55MM}$ genotype (slow metabolizers) was 14% while in controls it was only 10%. Without considering pesticide exposure, a higher OR was found among *PON1*$_{55MM}$ genotypes (adjusted OR = 1.45; 95% CI 0.87 – 2.40). When considering high chlorpyrifos residential exposure, the OR increased to 1.56 (95% CI 1.02 – 2.40) and when combining subjects with both high and low residential chlorpyrifos exposure, the resulting OR increased to 2.61 (95% CI 1.25 – 5.44).

As an extension of the previous PEG study, these investigators considered additional sources of ambient exposure and examined two additional variants, *PON1*$_{Q192R}$ and *PON1*$_{C\text{-}108T}$, which were also slow metabolizers (Lee *et al.*, 2013). Subjects included 287 PD cases and 440 controls. Subjects were all Caucasian and with a slightly greater portion being male among cases (56.1%) compared to controls (49.3%). The prevalence of the slow metabolizer variants (*PON1*$_{55MM}$, *PON1*$_{192QQ}$ or the *PON1*$_{108AA}$) was slightly higher in the cases at 14.6%, 51.3% and 26.4%, respectively, compared to controls at 11.1%, 45.3% and 24.8%. They focused specifically on 3 OPs, including chlorpyrifos. They did not find any association of PD risk between the *PON1*$_{C\text{-}108T}$ variants regardless of OP exposure; however, they did find a higher PD risk with the *PON1*$_{55MM}$ and *PON1*$_{192QQ}$ variants based on their OP exposure. The adjusted OR was clearly significant for chlorpyrifos exposure and *PON1*$_{55MM}$ (2.45, 95% CI 1.24 – 4.83). The adjusted OR for *PON1*$_{192QQ}$ and chlorpyrifos exposure was lower, but still significant (1.95, 95% CI 1.13 – 3.37).

In another PEG study conducted by Narayan et al. (2013), exposure to household pesticide and risk for PD was examined. As with previous PEG studies, PD cases (357) were more likely to be male and white (57.4% males and 80.5% white) with fewer white males among controls (807; 46.0% males and 69.9% white). Exposure was based on self-reported use of home and garden pesticide products along with DPR's product label database. Exposure was classified as either none or rare or frequent. Subjects were genotyped for *PON1*$_{L55M}$ and *PON1*$_{Q192R}$. The prevalence of the variants for these genotypes was not reported. The association between frequent pesticide use was significant (adjusted OR =1.47, 95% CI1.13 – 1.92), but even greater for frequent OP use (adjusted OR = 1.71, 95% IC = 1.21 – 2.41). When association with chlorpyrifos exposure was examined, the adjusted OR was 2.73 (95% CI 1.03 – 7.24), possibly due to the small number of cases and controls (9/9). When *PON1*$_{192QQ}$ genotype was considered, the adjusted OR for frequent use of OPs was 2.51 (95% CI 1.28 – 4.94) and for frequent organothiophosphate use the OR was 3.71 (95% CI 1.42 – 9.68). Since exposure for this study was assessed retrospectively, recall bias could have contributed to findings.

Table 7. Summary of Parkinson's Environment and Gene (PEG) Epidemiology Studies Examining Chlorpyrifos Exposure

Study	Pesticide/Exposure	Cases/ Controls	Adjusted Odds Ratio (95% CI)
(Gatto *et al.*, 2009) Residential cumulative ambient exposure	Chlorpyrifos Unexposed Ambient only Ambient + well water - all Low High	 186/210 115/90 67/41 25/21 42/20	 1.00 (reference) 1.42 (1.00-2.01) 1.63 (1.04-2.57) 1.05 (0.56-1.96) 1.87 (1.05-3.31)
(Manthripragada *et al.*, 2010) Residential average ambient exposure	*PON1-55* variants and PD LL LM MM	 159/180 144/148 48/35	 1.00 (reference) 1.04 (0.75-1.44) 1.45 (0.87-2.40)
	Chlorpyrifos – Residential ambient Low High	 93/74 88/74	 1.56 (1.06-2.31) 1.56 (1.02-2.40)
	Chlorpyrifos – Low/High Exposure *PON1-55* LL + LM *PON1-55* MM	 154/135 27/13	 1.48 (1.04-2.12) 2.61 (1.25-5.44)
(Lee *et al.*, 2013) Cumulative ambient residential and workplace exposure	Chlorpyrifos – Low/High Exposure *PON1-55* LL + LM *PON1-55* MM *PON1-192* RR+QR variants *PON1-192* QQ variants	 134/188 26/21 73/100 83/82	 139 (0.91-2.12) 2.45 (1.24-4.83) 1.48 (0.86-2.56) 1.95 (1.13-3.37)
(Narayan *et al.*, 2013) Self-reported household use for 4 age periods (16-24 yrs, 25-44 yrs, 45-64 yrs and ≥ 65 yrs)	Household Use of Pesticides Any – frequent OPs – frequent Chlorpyrifos – frequent	 161/303 83/121 9/9	 1.47 (1.13-1.92) 1.71 (1.21-2.41) 2.73 (1.03-7.24)
	Organophosphates – frequent PON1-192 QQ variants Organothiophosphates - frequent PON1-192 QQ variants	 28/19 16/7	 2.51 (1.28-4.94) 3.71 (1.42-9.68)
(Wang *et al.*, 2014) Ambient residential and workplace exposure	Chlorpyrifos Ambient residential Ambient workplace Ambient residential and workplace Mitochondrial disruptor OPs Ambient residential Ambient workplace Ambient residential and workplace	 46/88 31/57 39/64 69/138 53/84 110/168	 1.69 (1.06-2.69 1.94 (1.12-3.34) 1.92 (1.15-3.18) 1.7 (1.13-2.58) 2.22 (1.41-3.51) 2.23 (1.52-3.27)

Wang et al. (2014) evaluated in the associated of PD with ambient workplace and residential exposure in another population-based case-control PEG study which involved 357 cases and 752

controls. A positive association was found for PD and ambient residential exposure to chlorpyrifos (adjusted OR = 1.69; 95% CI 1.06 – 2.69). The association was stronger for PD and ambient workplace exposure to chlorpyrifos (1.94, 95% CI 1.12 – 34). They also grouped together OPs based on their mechanism of toxicity to see if there were any associations based on that. For OPs that caused mitochondrial disruption, which included chlorpyrifos, significant positive associations with PD were found with either residential or workplace exposure, but particularly with combined residential and workplace exposure (2.23, 95% CI 1.52 – 3.27). However, the strongest association with PD was with OPs that were carcinogenic (which did not include chlorpyrifos), especially with combined residential and workplace exposure (3.21, 95% CI 1.75 – 5.91).

There are a couple other case-control studies that were conducted outside California that examined the association of PD with exposure to chlorpyrifos along with other pesticides. In one case-control study involving pesticide applicators and their spouses from Iowa and North Carolina who participated in the Agricultural Health Study found positive associations with incident (i.e., sporadic) PD and personal application of pesticides, but none of the adjusted ORs were significant, except when cumulative days of use were greater than 397 days over a lifetime (Kamel *et al.*, 2006). When individual pesticides were examined, the adjusted OR for chlorpyrifos was clearly not significant (0.9, 95% CI 0.5 – 1.6). A case-control study in Texas found positive associations of PD with pesticide exposure, but only the exposure to rotenone was clearly significant (10.0, 95% CI 2.5 – 48.0) (Dhillon *et al.*, 2008). Exposure to chlorpyrifos was positively associated with PD (adjusted OR= 2.0; 95% CI 1.02 – 3.80). They also found positive associations of PD with industrial chemicals, but none of these were significant based on their 95% CI.

In a case-only study of pesticide handlers in Washington State (Nielson *et al.*, 2015), the levels of plasma α-synuclein were measured. α-Synuclein is a protein that aggregates in Lewy bodies which are considered a pathological hallmark of PD. They also measured blood ChEI and BuChE-CPF adducts as biomarkers of exposure and they found no association of BuChE-CPF adducts, blood ChEI or self-reported chlorpyrifos exposure with increased α-synuclein levels. They also looked at the association of plasma α-synuclein levels and the polymorphism of two PON1 genotypes, $PON1_{Q192R}$ and $PON1_{C\text{-}108T}$. They did find higher α-synuclein levels with the $PON1_{108T}$ allele and with more than 10 hrs exposure to a ChEI insecticide in the past 30 days, but neither had a clear dose response.

II.M.4.b. Animal Studies of Parkinson's Disease

As previously discussed in Section II.I, Behavior and Developmental Neurotoxicity in the December 2017 Draft TAC Evaluation, researchers observed significant reductions in the DA levels in the hippocampus, but not in the striatum of rat pups given chlorpyrifos in dimethyl sulfoxide (DMSO) during GD 17-20 at 1 and 5 mg/kg/day which are near the threshold for AChE inhibition (Aldridge *et al.*, 2005). The DA turnover was increased in the cerebral cortex, striatum and midbrain of the pups at 5 mg/kg, but not 1 mg/kg. The changes in DA levels and turnover were minor in pups exposed to these same doses on PND 1-4 (decreases in cerebral cortex, increases in striatum and midbrain) and no effects in DA levels were seen in pups exposed PND 11-14 at these doses, indicating a window of vulnerability closed in the second postnatal week. The investigators suggested that the differential sensitivity of the hippocampus

compared to the striatum indicate that oxidative stress was not a contributing factor in this dopaminergic developmental neurotoxicity since the striatum has a high concentration of DA which is considered an oxidative neurotransmitter. The studies are summarized in Table 8, below.

Table 8. Studies Evaluating Effects Related to Parkinson's Disease in Animals Exposed to Chlorpyrifos

Species, Sex, Age	Exposure Route & Duration	Effect	LOEL mg/kg/day	Ref.[a]
Rat, Pups GD 17-20	CPF s.c. daily in DMSO 1 or 5 mg/kg/day	↑ DA level in hippocampus	1	1
		↑ DA turnover	5	
PND 1-4	1 mg/kg/day	Minor ↑ DA level & turnover	1	
PND 11-14	5 mg/kg/day	No effect on DA level	--	
Rat, Pups PND1-21	CPF gavage in corn oil 0 or 1.5 mkd PND 1-7 → 3 mkd PND 8-14 → 6 mkd PND 15-21	PND 22: Change in ratio of nAChR subunits expression	1.5>6	2
		PND 50: ↑DOPAC and DA turnover, no effect on nAChR subunit expression		
Rat, pups PND 11-14	CPF s.c. daily in DMSO 0 or 5 mg/kg/day	↓ Dopaminergic neurons & ↑ neuroinflammation in substantia nigra	5	3
Mice, pups GD0–8 mos	CPF in diet, 0, 0.1, 1, or 10 mg/kg/day	↓ Brain AChE (30%), ↓ dopaminergic gene expression	10	4
		↑ gene expression of *UBC* and *Casp9*	0.1	
Rats, M Adults Age NR	CPF s.c. in olive oil 0 or 250 mg/kg	Day 2: ↑ DA turnover in striatum Day 7 & 15: ↓ 5-HT turnover in striatum Day 30: ↓ DA, 5-HT, NE & metabolites in nucleus accumbens	250	5
Mice, M 7-9 mos	CPF gavage in corn oil, 3X in 2 weeks, 75 mg/kg	No effect on gene expression of α-synuclein, DT or TH	75	6
Rats, M 11 wks	CPF s.c. in peanut oil, daily for 21 days, 3 or 10 mg/kg/day	↓Brain AChE (87%), ↑ expression of *Nptx2* in hippocampus	10	7
		↓ Brain AChE (42%), no effect on PD related gene expression	3	
Mice, M, 7-8 mos	CPF s.c in corn oil, 3X in 2 weeks 0, 25, 50 & 100 mg/kg	↓ activity in FOB, ↓ DA uptake, ↑ DOPAC, ↓ MTT activity	100	8
Mice, M 7-9 mos	CPF s.c. in corn oil, pretreated with MPTP 0 or 50 mg/kg	No additional ↓ TH or ↑ GFAP with CPF	--	9
Mice, M 10-12 wks	CPF s.c. in saline, 3X in 2 weeks, 0 or 80 mg/kg	Hind limb paralysis, neuro-degeneration & protein deposits in substantia nigra, ↑ biomarkers for oxidative stress in plasma & brain	80	10

a References: 1. (Aldridge *et al.*, 2005); 2. (Eells and Brown, 2009); 3. (Zhang *et al.*, 2015); 4. (Pallotta *et al.*, 2017); 5. (Moreno *et al.*, 2008); 6. (Kou *et al.*, 2006); 7. (Lee *et al.*, 2016); 8. (Karen *et al.*, 2001); 9.(Dodd and Klein, 2009); 10. (Devici and Karapehlivan, 2018).

Abbreviations: CPF = chlorpyrifos; s.c. = subcutaneous injection; DMSO = dimethyl sulfoxide; DA = dopamine; mkd = mg/kg/day; PND = postnatal day; DOPAC = 3,4 dihydroxyphenylacetic acid; nAChR = nicotine acetylcholine receptor; AChE = acetyl-cholinesterase; 5-HT = serotonin; NE = norepinephrine; DT = dopamine transporter; TH = tyrosine hydrolase; PD = Parkinson's disease; FOB = functional observational battery; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GFAP = glial fibrillary acidic protein.

Eells and Brown (2009) also examined the effects of chlorpyrifos given s.c. in corn oil to newborn rat pups at increasing doses from 1.5 mg/kg/day on PND 1-7, 3 mg/kg/day on PND 8-15 and 6 mg/kg/day on PND 16-21. On PND 22, the levels of DA and it's metabolites, 3,4 dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum, were not significantly different from the vehicle controls nor was the DA turnover (DOPAC/DA or HVA/DA) affected. However, on Day 50 DOPAC levels were elevated as well as the DA turnover. The also examined the dopamine transcription factors, *Nurr1* and *Lamx1b,* and the expression of genes involved in dopamine neurotransmission, including tyrosine hydrolase (TH), GTP cyclohydrolase, dopamine transporter (DT), vesicular monoamine transporter 2, and the nicotine acetylcholine receptor (nAChR) subunits, α6 and α7. TH is involved in DA synthesis and DT is involved in the uptake of DA into neurons. On Day 22, only the ratio of the nAChR subunits was altered (↓α7/α6). On Day 50, there was no difference in the ratio of these nAChR subunits or any other gene expression related dopamine neurotransmission.

Others have reported changes in the dopaminergic system in developing rats and mice at low doses. There was a significant reduction in dopaminergic neurons in rat pups receiving chlorpyrifos in DMSO s.c. at 5 mg/kg/day from PND 11 to PND 14 when examined on PND 30 and PND 60 ((Zhang *et al.*, 2015). Furthermore, there was increased immunostaining for cluster of differentiation protein 11b (CD11b) and glial fibrillary acidic protein (GFAP) in the substantia nigra indicating activation of microglia cells and astrocytes, respectively, indicating there was neuroinflammation. Specifically, there was an upregulation of the nuclear factor kappa B (NF-κB) p65 and p38 mitogen-activated protein kinase (MAPK) inflammatory signaling pathways. Pallotta et al. (2017) also found that long-term exposure in mice pups to chlorpyrifos in the diet during gestation through 8 months of age affected the expression of genes related to the onset of PD. No significant brain cholinesterase inhibition was seen at 0.1 and 1.0 mg/kg/day. Brain AChE inhibition was 80% at 10 mg/kg/day at 3 months and 30% at 8 months. At 3 months, down regulation of 4, 48 and 66 genes were seen at 0.1, 1 and 10 mg/kg/day, respectively. Of the four genes down-regulated at all doses, two were related to dopaminergic signaling(*Park2* and *Nr4a2*), one related to GABAergic signaling (*Gabbr2*), and one related to transmembrane transport activity (*Sv2b*). At 8 months of age, 2, 14 and 16 genes still had altered expression at 0.1, 1.0 and 10 mg/kg/day, respectively. Among the genes that had altered expression, more were upregulated than down regulated. Some genes related to the dopaminergic system were still downregulated at 10 mg/kg/day at 8 months, including *Park2*, *Atxn2*, and *DRD2*. The two genes that were altered (upregulated) at all three dose levels were *UBC* which is involved in maintaining ubiquitin levels under stress conditions and *Casp9* which is involved in apoptosis. Upregulation of UBC transcripts has been found in cerebrospinal fluid of PD patients.

Changes in DA levels and DA turnover have also been observed in adult animals exposed to chlorpyrifos. Moreno et al. (2008) administered a single dose of chlorpyrifos s.c. in olive oil to adult male rats (age not reported) at 250 mg/kg and then analyzed brain AChE levels as well as the levels of various monoamines, including, DA, serotonin (5-HT), norepinephrine (NE) and their metabolites DOPAC, HVA and 5-hydroxy-3-indolacetic acid (5-HIAA) in the striatum and the nucleus accumbens on Days 2, 7, 15 and 30 after dosing. The nucleus accumbens is a brain region involved in motivational function. Brain AChE was inhibited from 68% (Day 2) to 82% (Day 15) in the striatum and from 53% (Day 2) to 82% (Day 15) in the nucleus accumbens. No difference in DA, DOPA and HVA were seen in the striatum at any time. However, the DA turnover (i.e., DOPAC/DA and HVA/DA ratios) in the striatum was significantly increased on

Day 2. The 5-HT levels were also not affected in the striatum, but the 5-HT turnover (i.e., 5-HIAA/5-HT) was significantly reduced on Days 7 and 15. All of monoamine levels were significantly reduced in the nucleus accumbens on Day 30 including their metabolites, but only the HVA/DA ratio was significantly different.

In addition, changes in gene expression related to the dopaminergic system have been reported in adult animals. Kou et al. (2006) reported that there was no effect on the gene expression for α-synuclein, DT, and TH in the striatum of adult mice given chlorpyrifos in corn oil at 75 mg/kg by oral gavage. Usually the expression of both TH and DAT are reduced with PD. However, Lee *et al.* (2016) reported an increase in the gene expression of *Nptx2* in the hippocampus of adult rats when injected s.c. with chlorpyrifos in peanut oil at 3 or 10 mg/kg/day for 21 days. *Nptx2* encodes the neuropeptide, NPTX2, which is involved in long-term plasticity and response to a novel environment. Changes in its expression have been associated with PD. The expression of this gene was not affected at 3 mg/kg/day. Brain AChE activity was reduced to 58% and13% of controls at 3 and 10 mg/kg/day, respectively. Five other genes involved with receptor-mediated cell survival signaling pathways that have been associated with neurocognitive disorders were also increased at 10 mg/kg/day. These included *Bdnf* (Alzheimer's disease, Huntington disease, epilepsy, addiction), *Cort* (sleep disorders, reduced locomotor activity), *Crhbp* (reduced anxiety and bipolar disorder), *Npy* (addiction, compulsion behavior, anxiety) and *Pnoc* (anxiety and increased pain sensitivity).

Karen et al. (2001) reported effects on striatal dopaminergic pathways in adult male mice injected s.c. three times with chlorpyrifos in corn oil at 0, 25, 50 or 100 mg/kg/day over two weeks. Significant reductions in open field movement and rearing activity were seen in the mice receiving chlorpyrifos that were significant at 100 mg/kg. Reductions in these behaviors were also seen at 50 mg/kg, but the differences were not significant. There was no apparent effect on neurobehavior at 25 mg/kg. Dopamine (DA) uptake was only affected in mice receiving chlorpyrifos at 100 mg/kg. The ability to reduce the dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which is a measure of mitochondrial metabolic capability, was significantly reduced in the striatal only at 100 mg/kg. The dopamine metabolite, DOPAC, was only significantly increased in mice receiving chlorpyrifos at 100 mg/kg, but not at lower doses.

Dodd and Klein (2009) evaluated the effects of chlorpyrifos (50 mg/kg s.c in corn oil) in mice previously treated with, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 30 mg/kg i.p.) to determine if it increased the nigrostriatal damage induced by MPTP. They measured TH activity and the glial fibrillary acidic protein (GFAP) levels which is a biomarker of nervous system damage due to reactive gliosis (O'Callaghan and Sriram, 2005). Mice given MPTP only had reduced TH activity and increased GFAP levels. Mice given chlorpyrifos after pre-treatment with MPTP had no additional changes in TH activity or GFAP levels.

Devici and Karapehlivan (2018) claimed to have created a chlorpyrifos-induced Parkinson's model in mice by injecting them with chlorpyrifos in saline s.c. 3 times in 2 weeks at 80 mg/kg. They reported movement difficulties in the 1st week, walking difficulties in the 2nd week and hind limb paralysis and difficulties reaching food and water in the 3rd week. Histopathological examination of the substantia nigra (no other neuronal tissue examined) revealed neurodegeneration and deposits they described as Lewy bodies. However, they did not perform

immunochemical staining of the slides for α-synuclein to confirm that these deposits were Lewy bodies. These investigators did evaluate oxidative stress based on the total oxidant capacity (TOC), total antioxidant capacity (TAC), PON1 activity, lipid profile and total sialic acid (TSA) in plasma and brain. In the chlorpyrifos treated mice, TOC, LDL and TSA levels were elevated while the TAC, PON1, HDL levels were reduced compared to controls.

II.M.4.c. Mechanistic Studies of Parkinson's Disease

Apoptosis: Caughlan et al. (2004) reported that they induced apoptosis in rat cortical neurons with both chlorpyrifos and CPF-oxon. The mitochondrial dysfunction (based on reduced MTT activity) occurred at lower chlorpyrifos doses than apoptosis occurred suggesting that mitochondrial dysfunction precedes the apoptosis. CPF-oxon was only slightly more potent than chlorpyrifos indicating the apoptosis is unrelated to AChE inhibition. They also found embryonic (E17) neurons were more susceptible to chlorpyrifos, than postnatal (P0) neurons, but not CPF-oxon. They also observed that chlorpyrifos activated ERK1/2 and p38 MAP kinases and a sub-pool of c-Jun NH_2-terminal protein kinase (JNK). Blocking of these activations by various inhibitors suggests the ERK1/2 and JNK are acting as pro-apoptotic pathways, while p38 MAP kinase is acting as a compensatory survival mechanism to counteract chlorpyrifos neurotoxicity.

Oxidative Stress: Qiao et al. (2005) evaluated the potential of chlorpyrifos to cause oxidative stress in PC12 and SH-SY5Y cells. PC12 cells are rat pheochromocytoma cells that are immature neuronal precursor cells that can be induced to differentiate with nerve growth factor (NGF), developing axonal projections, electrical excitability, and increase the number of nicotinic AChE receptors (nAChRs). SH-SY5Y cells are human neuroblastoma cells which are also neuronal precursors that can be induced to differentiate with NGF. Chlorpyrifos at 30 to 100 μM caused a significant increase in thiobarbituric acid reactive species (TBARS) in undifferentiated cells. Initiation of differentiation by NGF did not increase TBARS with chlorpyrifos. Chlorpyrifos at these concentrations also caused a dose-dependent antimitotic effect on cells that was similar between undifferentiated and differentiating cells. Nicotine inhibited these antimitotic effects of chlorpyrifos when given at the same time. AChE inhibition was not measured in these cells, but Middlemore-Risher *et al.* (2011) observed AChE inhibition in rat primary cortical neurons at chlorpyrifos concentrations greater than 5 μM. Bagchi et al. (1995) reported an increase in leakage of lactate dehydrogenase (LDH) from PC-12 cells exposed to chlorpyrifos at 50 nM and higher which they considered an indicator of cellular damage and cytotoxicity. They also reported an increase DNA-single strand breaks (SSBs) in these cells at 200 nM. In vivo, rats given two doses of chlorpyrifos at 41 mg/kg by oral gavage 21 hrs apart had increased TBARs and DNA-SSBs in liver and brain homogenates. Although AChE activity was not measured in these rats this dose level was high enough that it should have caused significant AChE inhibition.

Garcia et al. (2005) provided evidence that glial cells are a target for chlorpyrifos in the later stages of neurodevelopment, but the effect of chlorpyrifos on glial cells in mature animals is less clear. Glial cells play an important role in neuroinflammation, therefore, activation of them could lead to generation of radical oxygen species (ROS) which could theoretically lead to PD (EFSA, 2017). In their a review of the function of glial cells in the adult brain, Jakel and Dimou (2017) found that the effect of ablation of glial cells depends on the glial population and whether the animal is healthy at the time of ablation. Microglia cells are immunocompetent and act like

phagocytes in the nervous system. Ablation of microglia was neuroprotective in Alzheimer's mouse model. On the other hand, ablation of astrocytes generally had negative effects in both healthy animals and animals with neurodegenerative diseases. Astrocytes have numerous functions with the brain, including maintenance of water and ion homeostasis, participation in the tripartite synapse and maintenance of the blood brain barrier. Ablation of oligodendrocytes also had primarily negative effects in healthy animals. There were no published studies of its effect in animals with neuropathological conditions.

Dopaminergic Signaling: Torres-Altoro *et al.* (2011) evaluated the effect of CPF-oxon on downstream effectors in the dopaminergic signaling pathway in mouse striatal slices *ex vivo* and in mice and rats in vivo. They observed in mouse striatal slices that CPF-oxon at 100 μM for 60 min caused hyperphosphorylation of certain sites in downstream effectors, DARPP-32 (dopamine and cAMP-regulated phosphoprotein of M_r 32 kDa) and GluR1 (glutamate receptor 1) subunit of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor. Hyperphosphorylation of these downstream effectors also occurs with D1 dopamine receptor agonists affecting trafficking, stability and striatal neuron excitability. In vivo, they found that mice injected s.c. with CPF-oxon at 30 mg/kg/day daily for 7 days had a 1.36-fold increase in the phosphorylation of striatal GluR1, but no hyperphosphorylation was seen in mice injected s.c. with CPF-oxon at 1 or 2.5 mg/kg for the same period. Hyperphosphorylation of the neurofilament, tau, by the cyclin-dependent kinase, Cdk5, has been associated with the loss of neuronal function and cell death and has been suggested as a biomarker for Alzheimer's disease. Cleavage of the Cdk5-activating neuronal cofactor p35 to p25 by calpain results in hyperactivation and redirection of Cdk-5 towards aberrant substrates such as tau. However, CPF-oxon administered to mice at 1 or 2.5 mg/kg s.c. for 7 days did not result in significant p25 generation. They also examined the electrophysiological changes in corticostriatal glutamatergic neurotransmission with CPF-oxon in rat brains *ex vivo* at 100 μM and found that CPF-oxon did not affect the miniature excitatory post-synaptic current (mEPSC) amplitude, but did cause a significant decrease in the inter-event interval of mEPSC events (i.e., increased the frequency). They suggested this indicates that CPF-oxon alters striatal neurotransmission by enhancing glutamate release from corticostriatal terminals in an action potential-independent manner.

Mitochondrial Dysfunction: Middlemore-Risher et al. (2011) reported that chlorpyrifos (1-20 μM) and CPF-oxon (0.005-20 μM) in rat primary cortical neurons resulted in dose-dependent increase in mitochondrial length and decrease in mitochondrial number and their movement in axons. These changes were seen at concentrations that did not inhibit AChE (5 μM CPF, 0.01 μM CPF-oxon) and were not blocked by cholinergic receptor agonists, such as atropine (muscarinic) and mecamylamine (nicotinic). However, these changes did not seem to affect mitochondrial viability or function based on mitochondrial membrane potential or ATP production. The mechanism of these mitochondrial changes is uncertain, but the authors postulated that it involved fusion and/or fission proteins and that reduced movement of mitochondria in the axons could lead to lead to compromised neuronal function and promote apoptosis.

Yamada et al. (2017) reported mitochondrial dysfunction in human induced pluripotent stem cells (iPSCs) exposed to chlorpyrifos at 30μM based on decrease in ATP levels and mitochondrial fragmentation. To investigate the possible role of the mitochondrial fusion protein,

mitofusin 1 (Mfn1), they performed knockdown of the *Mfn1* gene using a lentivirus-delivered shRNAs. Mfn1 is known to be involved in the fusion of mitochondria to form tubular networks which are a normal part of the cell homeostasis. With knockdown of *Mfn1*, chlorpyrifos reduced the expression of several neural differentiation marker genes in iPSCs. Specifically, knockdown of *Mfn1* increased phosphorylation of ERK and reduced the expression of *PAX6*, a key transcription factor that regulates neurogenesis. Based on these findings, these investigators proposed that chlorpyrifos reduced Mfn1 which lead to mitochondrial dysfunction evoking ERK phosphorylation, leading to suppression of *PAX6*.

Proposed Adverse Outcome Pathways for PD and Pesticides: After performing a systematic review of the literature associating exposure to pesticides and risk for Parkinson's disease, the European Food Safety Authority (EFSA) used the Adverse Outcome Pathway (AOP) conceptual framework to define biological plausibility in relation to epidemiological studies (EFSA, 2017). In this approach, they identified two AOPs for PD with molecular initiating events (MIEs) and key events (KEs). In AOP1, the MIE is the binding to Complex I. KE1 is the inhibition of Complex I with KE2 being mitochondrial dysfunction. The evidence used to build this model came from MPTP and rotenone. KE3 involves impaired proteostasis which refers to the homeostasis of proteins in space and time. Two major degradation systems that are part of this proteostasis are the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP). These systems are highly energy demanding and susceptible to oxidative stress. Exposure to pesticides known to inhibit UPS, such as benomyl, cyanazine, dieldrin, endosulfan, ferbam, metam, propargite, rotenone, triflumizole and ziram are thought to increase the risk for PD, especially among individuals with a genetic variant of the *SKP1* gene that is part of UPS pathway (Ritz *et al.*, 2016). Inhibition of the UPS pathway results in the accumulation of α-synuclein. Aggregation of α-synuclein can obstruct cellular transport, leading to impaired intracellular trafficking or trapping of cellular organelles, most importantly the mitochondria, in the wrong locations resulting in synaptic and cell dysfunction. KE4 is the degeneration of dopaminergic neurons that is associated with the presence of Lewy bodies that contain α-synuclein and other ubiquitin proteins. KE5 is the neuroinflammation that is the result of activation of glial cells due to the neural degeneration. Glial cell responses can be pro-inflammatory or anti-inflammatory depending on the activation states of the cells. Consequently, the neuroinflammatory response could increase or decrease the neurodegeneration in KE4. When the neural degeneration becomes severe enough it leads to the adverse outcome of Parkinsonism motor deficits. Motor deficits are the result of insufficient dopamine, leading to overactivation of both glutamatergic signaling and inhibitory GABAergic signaling. This results in an impaired feedback to the thalamus and cortex. The MIE for AOP2 is the redox cycling of a chemical initiated by electrons released by the mitochondrial respiratory chain. Evidence from paraquat and maneb was used to support this AOP. Paraquat does not inhibit Complex I, but it is a mitochondrial electron acceptor. KE1 is the generation of reactive oxygen species (ROS) in the mitochondria leading to mitochondrial dysfunction. The rest of the KEs are essentially the same as AOP1 with KE2, KE3 and KE4 being impaired proteostasis, neuroinflammation and dopaminergic neurodegeneration, respectively and the adverse outcome of PD.

II.M.5. Alzheimer's Disease

II.M.5.a. Human Epidemiological Studies of Alzheimer's Disease

There are no epidemiological studies that evaluated the association of Alzheimer's disease (AD) with exposure to chlorpyrifos specifically. However, a few studies evaluated the risk for AD with pesticide exposure in general. One of these was a prospective cohort study of elderly residents living in Cache County, Utah, in which the investigators performed baseline cognitive screening on 5,092 residents that were 65 years or older in 1995 and then re-evaluated them at 3, 7 and 10 years (Hayden *et al.*, 2001). For various reasons (prevalent dementia at start, death, moved away, refused participation, incomplete data) the number of subjects in the final analysis was reduced to 3,084. Of these, 572 reported pesticide exposure. Final diagnosis of dementia was assigned at consensus conferences using standard criteria. The pesticide exposure was self-reported based on interviews with questionnaires providing work histories and associated exposures. The adjusted Hazard Risk (HR) for those with any pesticide exposure was 1.38 (95% CI 1.09 – 1.76; p = 0.008). The adjusted HR for dementia in general in those with exposure to organophosphates was 1.31(95% CI 0.88 – 1.55), but was not statistically significant (p = 0.29). However, the adjusted HR increased when the diagnosis was limited to AD. Based on those cases, the adjusted HR for all pesticide exposure increased to 1.42 (95% CI 1.06 – 1.91; p = 0.02). When that was further narrowed to subjects with organophosphate exposure, the adjusted HR increased to 1.53 (95% CI 1.05 – 2.23) and was statistically significant (p = 0.03).

Yan et al. (2016) performed a literature review and meta-analysis of the epidemiological studies evaluating the risk for Alzheimer's disease with pesticide exposure. A total of seven studies were included in the meta-analysis. Most took place in other countries, including three in Canada, one in France, and another in Australia. The study conducted by Hayden et al. (2001) was one of two studies conducted in the US. The other study was conducted by French et al. (1985) and was a hospital-based case-control study. The overall OR for these 7 studies was significant at 1.34 (95% CI 1.08 – 1.67) without heterogeneity (p = 0.88, I^2 = 0.05%), indicating the selected studies were statistically homogeneous and, therefore, the results relatively reliable. Sensitivity analysis produced similar results indicating the relationships were relatively stable.

II.M.5.b. Animal Studies of Alzheimer's Disease

Three month old male Wistar rats were injected s.c with chlorpyrifos in peanut oil at 0, 2.5, 10, 18 or 25 mg/kg/day for 14 days and evaluated for effects on learning and memory in water maze 1 day and 14 days after the last dose (Terry *et al.*, 2003). Plasma cholinesterase activity was reduced at all levels with 30% reduction at the lowest dose. Decreased body weights and rearing and sniffing activity were seen at 10 mg/kg/day and higher. In the water maze test given one day after the last dose, significant longer time to the platform and distance to swim to get to the platform were seen at 18 and 25 mg/kg/day. There were no significant differences between groups with the 14-day recovery period before testing them in the water maze. The axonal transport was examined *ex vivo* with peripheral nerve axons from these rats after maze testing. Both anterograde and retrograde axonal transport were reduced at 10, 18 and 25 mg/kg/day one day after the last dose. A reduction in the axonal transport was still significant at 25 mg/kg/day with a 14-day recovery period. These investigators also tested the effect of a subthreshold dose of chlorpyrifos at 2.5 mg/kg/day for 5 days/wk for 4 weeks on grip strength. They found a significant reduction in grip strength after the end of this treatment regimen which was reversible with a 5-day recovery period.

Samsam *et al.* (2005) examined the learning ability and attention span of rats fed chlorpyrifos at low levels (0, 1, or 5 mg/kg/day) for one year with or without intermittent acute doses of chlorpyrifos (60 mg/kg initial dose and 5 doses at 45 mg/kg) in corn oil by oral gavage every 2 months. The chronic low doses facilitated learning based on lever press response for a food reward, but the acute high doses significantly reduced learning. The authors proposed that the facilitated response with chronic low exposure probably was the result of motor dysfunction although there was no direct evidence for this. The authors also evaluated sustained attention by having the rats perform a signal discrimination task (SDT). Two months after the end of dosing only the rats receiving acute doses of chlorpyrifos in addition to chronic chlorpyrifos at 5 mg/kg/day had reduced performances in the SDT. The authors concluded from these findings that permanent cognitive impairment occurs only in the presence of brain AChE inhibition followed by acute doses of chlorpyrifos high enough to elicit signs of toxicity.

The effect of several different dosing regimens with chlorpyrifos on the microtubule structures in brains of mice were examined by Jiang et al. (2010). One group of 4 female mice were injected s.c. with chlorpyrifos at 3 mg/kg/day for 14 consecutive days. Another group of 3 male mice received a single dose of CPF-oxon at 3 mg/kg. A third group of 2 female mice received with 6 doses of CPF-oxon at 1, 22, 48, 50 and 50.15 hrs. Oxon labeled tubulin at tyrosine 281 and serine 338 was found in the brains of mice receiving chlorpyrifos at 3 mg/kg/day for 14 days or single dose of CPF-oxon at 3 mg/kg based on the diethoxyphosphorylated tubulin residues. Six of 19 proteins involved in axonal transport were not detected in male mice treated with a single dose of CPF-oxon (heat-shock protein 84 kDa, alpha-internexin, Myosin Va, dynein cytoplasmic 1 light intermediate chain, cytoskeleton-associated protein 5 and microtubule-associated protein 2 isoform 1). These proteins were related to microtubule assembly, structure, stability and function. The microtubules from the oxon treated mice were shorter and narrower than controls. These investigators suggested that oxon exposure may have also triggered CaM Kinase II which could also have enhanced phosphorylation of proteins and contributed to the dissociation of the microtubules.

Salazar et al. (2011) examined the effects of an acute high dose of chlorpyrifos (50 mg/kg s.c.) on both transgenic (Tg) Swedish mice carrying the amyloid β precursor protein (AβPP) mutation for AD and wild type (WT) Swedish mice. The brain AChE inhibition in both Tg and WT treated mice was about 40% 72 hrs after treatment. These investigators evaluated the effect of chlorpyrifos on the neurobehavioral activity and learning in Tg and WT mice. The WT control mice exhibited significantly more climbing in the FOB than the other groups as well as resistance to removal from the cage. The control and treated Tg mice and the WT treated mice all had reduced touch and righting responses relative to WT controls. Differences in distance traveled in open field were reduced in Tg treated mice compared to Tg controls 7 months after treatment. Differences between control and treated WT mice were not significant. While learning acquisition in a water maze task was not affected in the Tg or WT mice 17 weeks after dosing, the retention of this learned task was significantly greater in the Tg treated mice compared to Tg control mice. Retention was slightly poorer in treated WT mice compared to control WT mice. In the rotorod test performed 19 weeks after treatment, Tg mice showed no significant increase in the time to fall between acquisition trial 1 and 2 while both the control and treated WT mice were able to spend significantly longer time on the rotorod in acquisition trial 2 compared to trial 1. Eight months after treatment, the amyloid β (Aβ) levels were significantly higher in brains of Tg treated mice compared to Tg controls. As expected, the amyloid β levels

Table 9. Studies Evaluating Effects Related to Alzheimer's Disease in Animals Exposed to Chlorpyrifos

Species, Sex, Age	Exposure Route & Duration	Effect[c]	LOEL mg/kg/day	Ref.[a]
Rats, M 3 months	CPF[b] s.c. in peanut oil daily for 14 days 0, 2.5, 10, 18 or 25 mg/kg/day	↓ BuChE (~30%) after single injection	2.5	1
		↓ BW, rearing & sniffing, ↓ axonal transport, transient	10	
		↓ performance in water maze, reversed with 14-day recovery	18	
		Irreversible ↓ axonal transport	25	
Rats, M 75 days 2 cohorts	CPF in diet, 1 year 0, 1 or 5 mg/kg/day +/- CPF at 45 mg/kg bimonthly by gavage in corn oil	**CPF diet only**: ↑ learning of LPR, no effect on SDT	1	2
		CPF diet + 6 acute doses: ↓ SDT 2 months after recovery	5	
		Control diet +6 acute CPF doses: ↓ learning of LPR & SDT	45	
Mice, F, 75-95 days	CPF s.c. for 14 days in corn oil/DMSO 0 or 3 mg/kg/day	↓ BuChE, CPO labeling of β-tubulin at tyrosine 281	3	3
M, 72 days	CPO s.c. once in EtOH 0 or 3 mg/kg	↓ AChE & BuChE (60-70%), ↓ body temp, motor activity, ↓ microtubule proteins (6/19), short & thin microtubules	3	
F, 127 days	CPO s.c. 6X in 50 hrs in EtOH 0 or 2.5 mg/kg	↓ AChE & BuChE in plasma (100%) & brain (45-50%), CPO labeling of β-tubulin serine 338	2.5	
Mice, M, 7 months Tg2576 (AD) & WT	S.C. once in olive oil, 0 or 50 mg/kg	**WT & Tg:** ↓ Brain AChE (40%), ↓ touch & righting **Tg only:** ↑ retention in treated vs. controls, ↓ rotorod time in both control & treated vs WT, ↑Aβ in treated vs. controls	50	4
Rats, M Age NR	CPF s.c. once in corn oil, 0 or 250 mg/kg +/- Aβ i.c.v. daily for 15 days	**+Aβ +/-CPF:** ↓ water maze performance, **+Aβ/-CPF:** ↓ MAP1A **-Aβ/+CPF:** ↓ MAP2	250	5
Rats, M & F 4 months Tg344-AD & WT	CPF s.c. daily in peanut oil/EtOH (90%/10%), 0, 3 or 10 mg/kg/day	**Tg +/- CPF:** hyperphosphorylated tau, amyloid plaques & vacuoles	--	6
		WT & Tg + CPF: ↓ BuChE (50%)	3	
		WT & Tg + CPF: ↓ BuChE (70%) ↓ NOR & BM & ↑ microglia (M) **Tg + CPF:** ↓ MWM tasks (M)	10	

a References: 1. (Terry *et al.*, 2003); 2. (Samsam *et al.*, 2005); 3. (Jiang *et al.*, 2010); 4. (Salazar *et al.*, 2011); 5. (Ruiz-Muñoz *et al.*, 2011); 6. (Voorhees, 2017).

b Abbreviations: CPF = chlorpyrifos; CPO = chlorpyrifos oxon; s.c. = subcutaneous injection; BuChE = butyrylcholinesterase; BW = body weight; LPR = lever press response for food reward; SDT = signal detection task; AChE = acetylcholinesterase; EtOH = ethanol; Tg = transgenic; AD = Alzheimer's disease; WT = wild type; Aβ = amyloid β; i.c.v. = intraecerebroventricular infusion; NR = not reported; MAP = microtubule-associated protein; NOR = novel object recognition; BM = Barnes maze; MWM = Morris water maze.

c Bolding denotes which effects are associated with which phase of the experiment, and are for organization purposes only.

were low in both control and treated WT mice. The investigators suggested the increase in treated mice may be due to the inhibition of acyl peptide hydrolase (APH) by chlorpyrifos which is a serine hydrolase involved in the clearance of amyloid β. The IC_{50} by CPF-oxon is approximately the same for APH and AChE around 20 nM (Casida and Quistad, 2005).

Ruiz-Muñoz et al. (2011) examined the effect of chlorpyrifos (250 mg/kg s.c.) in rats with and without subsequent intracerebroventricular (i.c.v.) infusions of Aβ for 15 days on learning and memory in a water maze test, on histological staining for Aβ deposits in the brain and on microtubule-associated protein (MAP) levels in the brain. There was no effect on performance in the classic water maze test on Days1-5 until the hidden platform was moved on Day 7. When that happened, the animals receiving the Aβ infusions with or without chlorpyrifos performed worse, although those receiving both chlorpyrifos and the Aβ infusions had the worst performance. The investigators suggested the difference was due to difficulty in developing new navigation plans and impaired cognitive flexibility or an impaired memory problem that was not detected in the early phase. No Aβ deposits or signs of cell death were found in any of the rat brains, but the Aβ infusions without chlorpyrifos caused reduced MAP1A levels in hippocampus and prefrontal cortex while chlorpyrifos with the Aβ infusions caused reduced MAP2 levels in the prefrontal cortex. MAPs can polymerize tubulin to form microtubules. MAP1A is related to spine plasticity while MAP2 is considered a dendritic marker. They interpreted these changes to indicate that chlorpyrifos and Aβ transiently induce a decrease in dendritic and synaptic connections.

Voorhees (2017) examined the effect of chlorpyrifos on the progression of AD in WT and transgenic (TgF344-AD) rats when injected s.c. at 0, 3 or 10 mg/kg/day for 21 days. BuChE was inhibited 50 and 70% in males and 75 and 90 % in females at 3 and 10 mg/kg/day, respectively. AChE activity was not measured. No overt cholinergic signs were seen, although the chlorpyrifos treated rats were more agitated as indicated by their tail writhing behavior. Very few WT male rats exhibited agitation even with chlorpyrifos exposure. Female WT and both sexes of TgF344-AD rats showed increased agitation with chlorpyrifos exposure. Based on performance in several types of tasks [novel object recognition (NOR) task, Barnes maze (BM), and elevated-plus maze (EPM)], no differences were seen in chlorpyrifos treated rats of either sex at 3 mg/kg/day or in female chlorpyrifos treated rats at 10 mg/kg/day compared to WT controls. Cognitive deficits were seen in the NOR and BM performances in TgF344-AD rats with chlorpyrifos at 10 mg/kg/day relative to WT controls at 6, 16 and 24 months with intermittent recovery at 9 and 12 months. No difference in EPM was seen with chlorpyrifos exposure in either WT or TgF344-AD rats. At 24 months, rats were also tested in the Morris water maze (MWM) and as with earlier time points only the males showed deficits. These deficits were seen in both WT and TgF344-AD rats receiving chlorpyrifos at 10 mg/kg/day. The neuronal damage (vacuoles in cortex and hippocampus) was seen in both sexes of TgF344-AD rats and was further exacerbated by chlorpyrifos exposure, especially in males at 10 mg/kg/day. Amyloid plaque deposition were seen in TgF344-AD rats at 12-24 months, but was not affected by chlorpyrifos exposure. Chlorpyrifos treatment had no effect on levels of either total tau or abnormally phosphorylated tau in TGF344-AD rats. However, neuroinflammation based on CD68 immunoreactivity that is a biomarker for microglia activation was seen with chlorpyrifos at 10 mg/kg/day that was significant in both WT and TGF344-AD rats compared to their respective controls. GFAP, a biomarker for astrocyte activity, was elevated in TgF344-AD control rats

when compared to WT rats, but was reduced in TgF344-AD rats receiving chlorpyrifos at 10 mg/kg compared to TgF344-AD control rats.

II.M.5.c. Mechanistic Studies of Alzheimer's Disease

In 1986, (Iqbal *et al.*) reported that the protein tau which stimulates the assembly of microtubules was abnormally phosphorylated in the brains of patients with Alzheimer's disease. Microtubule assembly was only observed in control brains, but not Alzheimer's brains. The Alzheimer's brains did not have any inhibitor of microtubule assembly or abnormality of tubulin. Assembly could be stimulated in the Alzheimer's brains with DEAE-dextran that mimics tau.

Prendergast et al. (2007) examined the immunoreactivity (IR) of microtubule-associated proteins in rat hippocampal slices exposed to CPF-oxon at 0.1-10 μM for 1-7 days which produced 15-60% AChE inhibition. Reduction in MAP2 IR were seen as early as 24 hrs even at CPF-oxon concentrations as low as 0.1 μM. The α-tubulin IR was not affected at any time point or concentration. Cell damage was also evaluated in these hippocampal slices using fluorescent microscopy. With fluorescent microscopy, injury to CA1 and CA3 pyramidal cells and dentate cells were seen 3 days after exposure at all concentrations. Effect of CPF-oxon (0.1-10 μM) on polymerization was also examined with purified bovine tubulin dimer. CPF-oxon reduced polymerization 60-70% in MAP deficient tubulin that was not concentration dependent, but with MAP-rich tubulin, the reduction in polymerization by CPF-oxon was 2-fold greater and was dose-dependent. Based on these findings, the investigators proposed that phosphorylation of MAPs lead to their destabilization which results in disassembly of microtubules.

Grigoryan and Lockridge (2009) exposed purified bovine tubulin (0.1mM) to CPF-oxon for 30 min. at 5-100 μM and then polymerized by at 1mM GTP to generate microtubules. At 5 and 10 μM, CPF-oxon inhibited polymerization with a reduction the number of microtubules and the microtubules were thinner and shorter. However, at 25 μM, CPF-oxon stimulated polymerization with an increase in the number microtubules and in their length compared to controls. At 50-100 μM CPF-oxon, aggregates were formed. The investigators suggested at lower concentrations CPF-oxon partially blocked polymerization, but at 25 μM CPF-oxon stabilized the microtubule structure. At 50-100 μM, CPF-oxon began to destabilize the microtubules by covalently binding to the tyrosine residues. Nanoimaging showed that CPF-oxon was noncovalently bound to 17 of 35 tyrosines in the unpolymerized α- and β-tubulin. Grigoryan et al. (2009) used LC/MS/MS mass spectrometry to confirm the identity of the oxon phosphorylated tyrosines in treated tubulin. Tyr 83 was the most extensively labeled residue (61%) on α-tubulin at high concentration tested (500 μM). On β-tubulin, Tyr 281 had the most labeling (34%). The tyrosines most commonly labeled with CPF-oxon were on the exposed surface of the tubulin.

In a review of the role of tau protein in the development of AD, Gendron and Petrucelli (2009) noted that tau is one of several proteins that can polymerize tubulin into microtubules. Other proteins that are known to polymerize tubulin include MAP1 and MAP2. Tau is primarily found in neuronal axons. The neurofibrillary tangles (NFT) associated with AD are also associated with other tauopathies, although in AD the NFT only occur in the neurons whereas with other tauopathies they can also occur in glial cells. Mutations in the gene *MAPT* that encodes tau are not genetically linked to AD, but other neurodegenerative diseases have been. The exact

neurotoxic species of tau has not been identified, but both a toxic gain of function (e.g., hyperphosphorylation of tau) and the loss of normal tau functions are thought to contribute to AD progression. Hyperphosphorylated tau has been found in AD brains and it has lower microtubule promoting activity in vitro. The hyperphosphorylation of tau may be the result of several mechanisms, including: 1) the activation of cdk5 via overexpression of p25; 2) the decreased expression of protein phosphatase 2A (PP2A) which can dephosphorylate tau; or 3) decreased expression of Pin1 which is a protein involved in the assembly, folding and transport of cellular proteins. Decreased levels of both PPA2 and Pin1 have been found in AD brains. Hyperphosphorylated tau is thought to interfere with axonal transport and lead to synaptic damage either by causing microtubule disassembly and loss of tracks for axonal transport or by displacing cargo on tracks by binding to kinesin motor proteins that move cargo in the anterograde direction. Synaptic loss is an early event in AD and is more strongly associated with cognitive declines than NFT. NFT may initially be formed as a protective mechanism to sequester hyperphosphorylated forms of tau, but may eventually contribute to neuronal death by acting as physical barriers in the cytoplasm, displacing organelles and further interfering with axonal transport.

Morfini et al. (2009) proposed that defects in axonal transport are common in many adult-onset neurodegenerative diseases (AONDs) through different pathways. A common characteristic of these AONDs is the age-dependent decline in neuronal function which is initially associated with loss of synaptic activity rather than neuronal cell death that is a late event in the disease process. Axonal transport is essential for proper axonal and synaptic function because axons lack protein synthesis and the distance from cell body to synapses can be large. Microtubule-based motor proteins called kinesins transport organelles including mitochondria, synaptic vesicles and axolemmal precursors in an anterograde direction (from cell body to synapse) while cytoplasmic dynein acts as a motor in the retrograde direction carrying degradation products from the synapses to cell bodies. The phosphorylation of these motor proteins regulates axonal transport. Multiple kinases regulate the phosphorylation of these motor proteins and many of these are increased in AONDs indicating aberrant protein phosphorylation. Genetic mutations in these motor proteins have resulted in neuropathies that can vary depending on which subunit of the motor protein is mutated. However, most AONDs are not associated with genetic mutations in these motors. Instead, abnormal protein kinases and aberrant protein phosphorylation are considered the major hallmarks of AONDs. Studies with MPP^+ found that retrograde transport was increased while anterograde transport was reduced, suggesting that a proper balance in anterograde and retrograde transport are also necessary for neuronal function.

More recently there has been research suggesting that misfolding of proteins and disruption of the retromer complex are common mechanisms in neurodegenerative diseases (Tyson *et al.*, 2016; Sweeney *et al.*, 2017; Victoria and Zurzolo, 2017). Its role is closely related to proteostasis and axonal transport. Misfolding of proteins is a common event and removal of these misfolded proteins involves several systems. The ubiquitin proteasome system (UPS) is responsible for the removal of monomeric misfolded proteins while the autophagy-lysosomal pathway (AL) is responsible for removing oligomers of misfolded proteins to lysosomes. Deficiencies in the retromer complex can cause lysosomal deficiencies. The retromer is a pentameric complex of vacuole sorting proteins and sorting nexins that are responsible for sorting the endosomal compartments and depending on their on its cargo and their interactions with other complexes

directs them to the Golgi apparatus for recycling or to lysosomes for degradation. Mutations in the proteins forming the retromer have been associated with familial forms of AD and PD.

II.M.6. Conclusion

Exposure to chlorpyrifos has been associated with neurodegenerative conditions such as OPIDN, PD and AD that may occur through shared mechanisms, such as misfolding of proteins, disruption of axonal transport, and mitochondrial dysfunction. Based on animal studies, AD could occur with repeated exposures to chlorpyrifos (3-10 mg/kg/day) through hyper-phosphorylation of tau and other proteins involved in axonal transport. It is important to note that significant RBC and brain AChE inhibition would also occur at these same dose levels. Hyperphosphorylated tau and MAP proteins are thought to lead to synaptic damage either by loss of microtubule tracks for axonal transport or by displacing cargo on tracks by binding to kinesin motor proteins that move cargo in the anterograde direction. NFTs are formed from hyperphosphorylated tau and may initially be a protective mechanism to sequester the toxic (hyperphosphorylated) form of tau, but these NFTs may eventually contribute to neuronal death by acting as physical barriers in the cytoplasm, displacing organelles and further interfering with axonal transport. Synaptic loss is an early event in AD and is more strongly associated with cognitive declines than NFTs. The plaques are also from the accumulation of misfolded Aβ. By itself, chlorpyrifos does not appear to increase Aβ levels, but in Tg-AD mice and rats treated with chlorpyrifos, the Aβ levels were higher than in the Tg-AD controls. Hyperphosphorylation of α-synuclein can also lead to its misfolding and formation of aggregates referred to as Lewy bodies that are the hallmark of PD. In one epidemiological study in handlers they saw no increase in α-synuclein levels nor did they find an increase in α-synuclein gene expression in mice treated with chlorpyrifos at 75 mg/kg. Chlorpyrifos was associated with significant inhibition (69%) of the mitochondrial respiratory enzyme, Complex I, in hens at 150 mg/kg. Mitochondrial dysfunction can lead to impaired proteostasis through disruption of the major protein degradation systems including UPS and ALP which are highly energy demanding. The impaired proteostasis can result in protein misfolding and aggregation which can then interfere with axonal transport and lead to neurodegeneration from organelles, especially mitochondria, and nutrients not being where they are needed. Neuroinflammation in response to protein aggregates and neuronal damage can contribute to further neuronal damage. At supralethal doses chlorpyrifos causes significant inhibition of NTE (> 70%) that can cause further mitochondrial dysfunction by disrupting calcium homeostasis leading to its accumulation in the mitochondria which increases its permeability. There may also be some disruption of dopaminergic signaling and gene expression at low doses of chlorpyrifos which could lead to PD later in life, but this has not been demonstrated in animals yet. Chlorpyrifos may also contribute to AD by the inhibition of another serine hydrolase, APH, which is involved in the clearance of Aβ. At higher doses, oxidative stress related to the AChE inhibition may also contribute to mitochondrial dysfunction.

Collectively, it appears that high doses/exposures of chlorpyrifos are associated with various types of neurodegeneration. At present, there is no evidence suggesting that chlorpyrifos-related neurodegeneration occurs at lower doses, such as those below the level that inhibits AChE.

II.N. Additional Effects of Chlorpyrifos

II.N.1. Chlorpyrifos Effects on the Respiratory System

In its findings on the December 2017 Draft TAC Evaluation, the Office of Environmental Health Hazard Assessment (OEHHA) suggested that the respiratory effects associated with chlorpyrifos exposure be considered when establishing potential critical toxicity endpoints. OEHHA cited published epidemiological data from the Agricultural Health Study (AHS) that associated exposure to certain OPs with wheeze in exposed occupational and bystander cohorts (Hoppin *et al.*, 2006b). As such, HHA re-evaluated the public and occupational health studies that investigated respiratory outcomes.

Hoppin et al. (2006) showed a dose-related increase in the odds ratio of wheeze episodes with increasing days of chlorpyrifos application. However, the authors did 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 AHS results by the same investigators (Hoppin *et al.*, 2017), are summarized in Table 10.

Respiratory effects were reported in four studies. However, in each case the data were not adequate for the development of PoDs because of uncertainties intrinsic to the assignment of the dose levels. Nevertheless, the review provided evidence to support the role of chlorpyrifos as a putative respiratory toxicant.

Table 10. Published Studies Reviewed to Evaluate Potential Respiratory Effects Related to Occupational and Bystander Exposure to Chlorpyrifos

Reference	**Type of Study/Design**	**Key Findings**
(An *et al.*, 2014)	Worker exposure study in China; dermal (DE) and inhalation exposures (IE) of CPF applicators (backpack pump with EC 48% CPF) were evaluated using personal dosimetry for sample collection and gas chromatography for quantification; maize fields of increasing heights (3 levels: 62, 108, and 212 cm) and increasing levels of personal protective equipment (PPE: 1 or 2 additional layers of cotton garment and cotton gloves; base included socks, rubber boots, and cotton inner/outer hats) were evaluated; estimated exposures (using DE and IE data) were compared to an acceptable exposure factor (AE) = 0.01 mg/kg day (per UK-CDR doc 2009) x 61.26 kg to calculate a margin of safety (MOS) (≥ 1 considered "safe"); safe work time (SWT) was also estimated.	Exposures increased with increasing crop height whether or not additional PPE was used (1 or 2 layers of additional garment and gloves). Decreases were observed for corresponding MOS and SWT parameters. IE below LOD for all but tallest crops. No data were reported that could be used to develop a PoD based on respiratory effects.

Reference	Type of Study/Design	Key Findings
(Bouchard *et al.*, 2010)	A multi-compartment model was developed to describe the human "biodisposition kinetics" of CPF and its metabolites 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) and alkyl phosphates (APs) diethyl thiophosphate (DETP) and diethyl phosphate (DEP); the model was validated using levels of the above species in human blood and urine; biological reference values (BRVs) (safe levels of absorption or exposure (primarily dermal) for workers) based on a repeated-dose NOEL for AChEI (0.1 mg/kg/day) were proposed.	BRVs were proposed for 3,5,6-TCP and APs in 0-24 and 0-48 hour urine pools based on an 8 hour exposure period at an absorbed dose level of a 0.08 mg/kg (0.1 mg/kg x 0.798 "the oral absorption fraction" and a dermal absorb rate = 0.04 $hour^{-1}$). No data were reported that could be used to develop a PoD based on respiratory effects.
(Burns *et al.*, 1998)	A continuation of a retrospective, case-control study of Dow employees that worked in CPF manufacturing areas between 1977 and 1994 (n = 496); the study included age-matched controls (n = 911); exposed cohort grouped into four exposure classifications (negligible: < 0.01 mg/m^3 or negligible potential dermal, low: < 0.03 ≥ 0.01 mg/m^3 or low potential dermal, moderate: < 0.2 ≥ 0.03 mg/m^3 or moderate potential dermal, and high: ≥ 0.2 mg/m^3 or high potential dermal); the study involved a questionnaire and a review of medical records; Blood cholinesterase activity date were available for all but 32 cases.	Most case were classified as having had moderate exposure (n =345) while a single case was classified as having had high exposure. The following respiratory effects with odds ratios (ORs) > 1 included): Acute respiratory infections (RI) (OR 1.39; CI 1.08 to 1.79) Acute RI (OR 1.49; CI 1.08 to 2.05) Other diseases of upper respiratory tract (OR 1.07; CI 0.76 to 1.50) Chronic obstructive pulmonary disease and allied conditions (OR 1.41; CI 0.95 to 2.09) Other diseases of respiratory system (OR 2.80; CI 1.18 to 6.65) The following effects had ORs > 1* but no continuous response when correlated with exposure level, mean ChE activity, or minimum ChE activity: Diseases of the ear and mastoid process *Mean ChE activity ≤ 50% (highest dose group) had an OR = 0.30 Acute respiratory infections While relevant respiratory effects data were reported, they were not adequate for the development of a PoD because of the uncertainties intrinsic to the assignment of the dose classifications.

Reference	Type of Study/Design	Key Findings
(Byrne *et al.*, 1998)	Residential exposure study designed to assess oral, dermal, and inhalation pathways after crack and crevice and spot treatment with CPF (0.5% water emulsion; 663 to 718 mL or 3.32 to 3.94 g) in 3 occupied, single family, multi-room houses (Ind., IA); 2 adult volunteers per house observed label recommendations about access to treated areas but otherwise followed normal routines; samples collected for analysis included urine (day -1 to +10; 3,5,6-TCP and creatinine (CR)), air (day 0 to +10; CPF), floor deposition pads (CPF), and dislodgeable residues on hard toy surfaces and carpet (CPF).	There was variability in the timing and magnitude of average peak air concentrations for the 3 houses: Average peak concentrations /Day (µg/m^3/day): 0.301/1, 0.903/6, 0.669/2 There was variability in the loading of deposition pads between rooms and between houses. Pre-exposure 3,5,6-TCP in urine raged from 0.04 to 0.35 µg/kg/day 11-day cumulative excretion of 3,5,6-TCP in urine ranged from 0.01 to 0.40 µg/kg/day Average daily excretion of CPF-equivalents in urine ranged from 0.001 to 0.037 µg/kg/day Estimates of cumulative (respiratory, dermal, and oral) absorbed doses by children ranges from 0.26 to 2.10 µg/kg or 0.26 to 2.1% of the NOEL used for comparison (100 µg/kg/day; plasma ChEI). Corresponding MOEs were 48 to 385. No data were reported that could be used to develop a PoD based on respiratory effects.
(Callahan *et al.*, 2014)	Prospective, cohort study conducted during cotton season (~10 months duration) in Cairo, Egypt; cohorts included pesticide (CPF, etc.) applicators (18 years old or less; average = 15.6) (n = 38) and non-applicator controls (18 years old or less; average = 15.4) (n = 24); end-points included 3,5,6-trichloro-2-pyridinol (TCPy) levels in urine (days 73, 146, 269), pulmonary function testing with spirometry (2 assessments; forced expiratory volume (FEV) and forced vital capacity (FVC)), and self-reported wheezing.	There was no significant correlation between TCPy levels in urine and changes to FEV and FCV measurements between groups or between assessments. Wheeze ORs for applicators were (unadjusted/age-adjusted): Day 146- 1.66 (CI 0.54 to 5.13)/1.71 (CI 0.55 to 5.36) Day 269-3.40 (CI 1.02 to 11.32)/3.27 (CI 0.97 to 11.08) While respiratory effects data were reported, they were not adequate for the development of a PoD because of a lack of dose data and uncertainties arising because of insufficient study power.
(Eddleston *et al.*, 2007)	Clinical review of the effects of acute poisoning by organophosphates (OPs) and the effectiveness of standard clinical interventions.	Respiratory infections can result from acute OP poisoning but may be the result of the need for ventilation. No data were reported that could be used to develop a PoD based on respiratory effects.

Reference	Type of Study/Design	Key Findings
(Fieten *et al.*, 2009)	A retrospective, cross-sectional study conducted in 2007 in Costa Rica; exposed (pesticides) (n = 69 plantain plantation workers) and unexposed cohorts (n = 58 banana plantation workers); study used a questionnaire and included spirometric evaluations.	No significant differences were observed between exposed and unexposed cohorts for FVC, FEV, or FEV/FCV ratio. ORs > 1 were observed for CPF and the following effects (all/weighted after stratification for smoking/non-smokers only): wheeze 2.7/3.5/6.7 shortness of breath 2.2/2.5/2.6 chronic cough 1.7/1.7/1.3 ORs for wheezing consistently increased with increased dose estimates for nonsmoking women. While respiratory effects data were reported, they were not adequate for the development of a PoD because of a lack of dose data.
(Gao *et al.*, 2014)	Worker exposure study in China; dermal exposures of CPF applicators (backpack pump with formulations containing 30 to 48% CPF) were evaluated; sample collection included a sorbent tube, skin swipes, and garment samples; gas chromatography was used for quantification; maize fields of increasing heights (3 levels: < 80, 80-130, and >130 cm); workers wore (pg. 637) " underwear, long pants, a long-sleeved shirt, cotton socks, rubber shoes, two-layer gloves, eight layers of gauze (20 × 40 cm) on the head, a half-facemask and a wide-brimmed hat to shield the head and neck from downward drift. Because pesticides could reach the body via openings in garments (e.g. unbuttoned shirts, unzipped suits, loose cuffs), it was ensured that shirts were fastened at the neck, that sleeves covered the gloves and that trouser legs covered the outside of the shoes".	Dermal exposures increased with increasing crop height and decreased with increased experience and increased layers of clothing. The inhalation exposures for mixers were higher than that for applicators. Dermal and inhalation exposures varied with the type of formulation used. No data were reported that could be used to develop a PoD based on respiratory effects.

Reference	Type of Study/Design	Key Findings
(Hoppin *et al.*, 2002)	Agricultural Health Study (AHS) study of pesticide applicators in IA (commercial applicators, farmers, family members) and NC (commercial applicators, farmers); 52000 applicators from 1994 to 1997. Two questionnaires were collected – one at certification enrollment and the second questionnaire was mailed (with 44% return rate); frequency of wheezing or whistling in the past year was analyzed in relation to modeled-exposures for dose-response assessment.	Of the 20,468 applicators, 19% reported at least one episode of wheezing and 5% reported diagnosed asthma or atopy. NC residents, smokers were more likely to report wheeze Total years-of-pesticide application was not a factor. Exposure was modelled and no estimates were presented. Total days of organophosphate use had not effect on elevation of wheeze risk. OR for wheeze in chlorpyrifos users was 1.12 (1.01 to 1.25) ORs for wheeze increased with increase in frequency of use <5 uses: OR 1.01 (CI 0.86 to 1.18) 5-9 uses: OR 1.33 (CI 1.13 to1.57) 10-19 uses: OR 0.91 (CI 0.71 to 1.15) ≥20 uses: OR 1.61 (CI 1.12 to 2.31) While relevant respiratory effects data were reported, they were not adequate for the development of a PoD because of the uncertainties intrinsic to the assignment of the dose classifications.
(Hoppin *et al.*, 2006b)	Cross-sectional AHS study of commercial pesticide applicator (not farmers or their family members) from IA; Commercial applicators that were certified as private applicators were considered as farmers, and not included in this analysis; 2255 participants from 1993-1997; data collected using self-administered questionnaires; exposures were modelled based on self-reported average number of days applied per year; exposure was modelled and presented as "number of days pesticide used in a year"	OR for wheeze in chlorpyrifos users was 1.47 (1.09 to 1.99) OR for wheeze increased with increase in frequency of chlorpyrifos use: <5 uses: OR 1.00 (CI 0.56 to 1.80) 5-9 uses: OR 1.10 (CI 0.58 to 2.08) 10-19 uses: OR 0.77 (CI 0.39 to 1.49) 20-39 uses: OR 1.96 (CI 1.05 to 3.66) ≥40 uses: OR 2.40 (CI 1.24 to 4.65) Authors refer to experimental evidence that airway hyperactivity occurs by decreasing neuronal M2 receptor function independent of AChE inhibition. While relevant respiratory effects data were reported, they were not adequate for the development of a PoD because of the uncertainties intrinsic to the assignment of the dose classifications.
(Hoppin *et al.*, 2006a)	Comparison of commercial applicator and farmer data from 2002 and 2006 AHS study publications.	No relevant data were reported that could be used to develop a PoD based on respiratory effects.
(Lee *et al.*, 2002)	CA Pesticide Air Monitoring data modelled to estimate CPF exposure levels.	No data were reported that could be used to develop a PoD based on respiratory effects.

Reference	Type of Study/Design	Key Findings
(Munoz-Quezada *et al.*, 2017)	Retrospective study to evaluate exposure and health status in Chilean farm workers (n=207); agricultural and non-agricultural workers were included.	47% of respondents reported using CPF. OP poisoning symptoms were reported. PPE were not followed in many cases. No quantitative exposure data were reported. No data were reported that could be used to develop a PoD based on respiratory effects.
(Perera *et al.*, 2005)	Prospective bystander exposure study; included 459 pregnant women in urban setting; personal air samples and blood specimens were collected and analyzed for OPs including CPF.	CPF detected in air samples and in 74% of the blood samples collected from mothers and newborn infants. An association was found between levels of OPs in umbilical cord and decreased infant birth weight and length. Birth weight decreased by 42.6 g and length by 0.24 cm for each log unit increase in cord plasma CPF levels. Respiratory effects were not reported. No data were reported that could be used to develop a PoD based on respiratory effects.
(Putnam *et al.*, 2008)	Simulated exposure study; respiratory and dermal exposure of golfers to CPF following application on turf grass was evaluated; CPF was applied at the maximum US EPA-approved rate; 8 volunteers (4 for dosimetry measurements and 4 for biomonitoring) played 18-holes of simulated golf over 4 hours; the inhalation dose was measured by personal air samplers; urine TCP levels were measured for biomonitoring; CPF exposure levels were estimated.	The dermal route was the dominant exposure pathway. No respiratory effects were studied. No data were reported that could be used to develop a PoD based on respiratory effects.
(Raanan *et al.*, 2015)	Prospective, population-based Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study; 526 of 601 enrolled pregnant women with live-born children; mothers and children (at 5 to 7 years of age) were evaluated for respiratory symptoms: 3 DEP metabolites were measured in urine samples of mothers (twice during pregnancy) and children (at ages 0.5, 1, 2,3.5 and 5 years); the relationship between DEP metabolites in urine (mother and child) and respiratory symptoms in children was evaluated.	OR for DEP metabolites in children's urine was 2.35 (1.27 to 4.34). Levels of DEP metabolites were associated with increased odds of reported respiratory symptoms 5 to 7 years later (OR 1.61 (CI 1.08 to 2.39)). Postnatal exposure to OPs over the course of childhood was associated with ORs > 1 of reported respiratory symptoms in children assessed at 5 and 7 years of age. While relevant respiratory effects data were reported, they were not adequate for the development of a PoD because of the uncertainties intrinsic to the assignment of the dose classifications.

II.N.2. Chlorpyrifos Effects on Metabolism and Obesity

As recommended at the January and March 2018 SRP hearings, HHA reviewed recent studies investigating potential association between organophosphate exposure and preconditions for Type 2 diabetes, obesity, and other metabolic disorders. Evidence from animal studies suggests that exposure to chlorpyrifos or organophosphate pesticides in general may disrupt metabolic regulation of glucose metabolism and insulin, with potential implications for the development of metabolic disorders and obesity in later life (Slotkin *et al.*, 2005; Lassiter and Brimijoin, 2008; Seidler and Slotkin, 2011; Reygner *et al.*, 2016; Fang *et al.*, 2018). However, the evidence from human studies is incomplete. Below is a summary of selected human and animal studies.

II.N.2.a. Human Studies on Metabolism and Obesity

In a prenatal study that involved 268 newborns in France, the level of the non-specific OP dialkyl phosphate (DAP) metabolites in maternal urine was found to correlate with the insulin level in cord blood serum (Debost-Legrand *et al.*, 2016). In a cross-sectional study involving 2227 adults in the 1999-2008 NHANES datasets, individuals with detectable urinary DAP levels were found to have higher diastolic blood pressure, lower HDL, and higher triglyceride than those below detection (Ranjbar *et al.*, 2015). However, no human study has shown the direct connection between early-life exposures to chlorpyrifos and later-life effects.

As summarized in Section II.K.1. Biomarkers of Human Chlorpyrifos Metabolism in the December 2017 Draft TAC Evaluation, DAPs metabolites are considered general metabolites of all OP-containing compounds in the environment. Because each urinary metabolite has multiple sources, the presence of any DAP metabolite in urine may result from exposure an O,O-diethyl pesticide or an environmental degradate, but cannot correlated to exposure to a specific active ingredient (Barr and Angerer, 2006).

A few human studies have also investigated whether developmental susceptibility to chlorpyrifos and OPs may vary with genetic polymorphisms. Paraoxonase 1 (PON1), a multifunctional enzyme that is involved in antioxidant defense, plays an important role in detoxification of chlorpyrifos and other organophosphate pesticides. Specifically, the PON1192 genotype has been shown to affect catalytic efficiency of the enzyme (Holland *et al.*, 2015). As part of the CHAMACOS cohort, 373 Mexican-American children in an agricultural community in California were analyzed for PON1 genotypic variations. The PON1192 genotype was found to link to higher odds of childhood obesity at the age of two (Huen *et al.*, 2013). However, it was unclear whether exposure to chlorpyrifos or OPs in general played a role in causing obesity in the genetically susceptible population.

Recently, the gut microbiome has been studied as a potential target for the diabetogenic effect of OPs. The gut microbiota can metabolize OPs into acetic acid, which is then converted into glucose by gluconeogenesis in the intestine and liver and accounts for glucose intolerance (Velmurugan *et al.*, 2017). A recent study in rural India showed a correlation between fecal esterase activity and self-reported exposure to OPs in humans (Velmurugan *et al.*, 2017). The same study also demonstrated a link between the fecal acetate and plasma OP level in diabetic individuals. Yet, it was unclear whether the glucose intolerance was caused by metabolic change in the gut microbiota at early life stage in these individuals.

II.N.2.b. Gestational or Neonatal Animal Studies on Metabolism and Obesity

In some animal studies there are indications that chlorpyrifos exposure may lead to metabolic disorders and obesity. Rats treated in utero through weaning showed increased body weights, increased fat, decreased insulin receptors, some body weight changes, and some evidence of hyperglycemia and hyperinsulinemia at doses of chlorpyrifos equal to or greater than 1.0 mg/kg/d (Reygner et al, 2016; Lassiter and Brimijoin, 2008). Neonatal rats treated on PND 1-4 showed increased insulin, cholesterol and triglycerides, factors that the authors associate with metabolic changes and cardiovascular disease later in life (Slotkin et al. 2005). Pups treated in utero exhibited different effects on gluconeogenic stimulation that again according to the authors may have long term effects on cardiovascular and liver function (Seidler and Slotkin, 2011). The majority of studies on energy balance and metabolism were performed in adult rats or mice, with most shoring effects on various aspects of energy metabolism, including increased body weights, affected total cholesterol, triglycerides, the insulin and leptin-signaling pathways, oxidative stress, and changes to gut microflora. A summary of pertinent studies is found below.

Slotkin et al., 2005. This study was performed to examine whether male Sprague-Dawley rats treated neonatally show the two main risk factors for type 2 diabetes and atherosclerosis (hyperinsulinemia and hyperlipidemia) as adults. Male pups were treated by subcutaneous injection (s.c.) at 0 (DMSO 1 ml/kg) and 1.0 mg/kg/d PND 1-4 (8/sex/dose), then pups were weaned at PND 21. There were no effects on pup growth, viability, body weight, or plasma levels of nonesterified free fatty acids or glycerol. The authors noted increases in cholesterol and triglycerides in fed and fasted animals but lipids, glucose concentrations, and percent of glycosylated hemoglobin and hemoglobin were within the normal range for males and females. Males (fed) had markedly increased insulin (returned to normal in fasted animals). Metabolic effects were more prevalent in males than females.

Lassiter and Brimijoin, 2008. This study was designed to examine the effects of chlorpyrifos on rat pup developmental neurotoxicity and weight gain after exposure in utero through weaning. Pregnant Long-Evans rats were treated by gavage at 0 (corn oil), 1.0, 2.5 and 4.0 mg/kg/d from GD 7 through PND 21. There were no maternal effects on body weight or clinical signs at termination. Body weights were significantly increased in males from PND 51 to 100 (maximum of 10.5% on PND 72). The authors also noted a 12% increase in male body volume and a decrease in specific gravity, and ascribed the change to increased fat, as it is a less dense tissue. Although not significantly different for treated versus control, the authors noted that leptin production was disrupted or clearance was increased in the treated animals versus controls, potentially leading to increased body weight gain in sexually mature animals.

Seidler and Slotkin, 2011. An investigation was performed to examine in utero and neonatal/perinatal chlorpyrifos exposure and disruption to β-andrenergic receptor mediated signaling associated with hepatic gluconeogenesis. Effects of chlorpyrifos treatment during different stages of early development on norepinephrine (NE) levels in liver were measured during adolescence and adulthood. Sprague-Dawley dams were treated s.c. with 0 (DMSO), 1 or 5 mg/kg/d during GD9-12 or 17-20. Neonatal treatment was PND 1-4 at 1.0 mg/kg/d or PND 11-14 at 5.0 mg/kg/d. Animals were then tested on PND 30 or PND 30 and 60 for norepinephrine (NE) in heart and liver. GD 9-12 treated pups showed statistically significantly increased NE in heart and liver on PND30. GD17-20 treated pups showed significantly decreased NE on PND 60

at 5.0 mg/kg/d in liver and at 1.0 and 5.0 mg/kg/d in heart. PND 1-4 and PND 11-14 treatment groups showed no effects on NE levels. Overall there were two distinct windows of treatment with opposite effects: early gestation exposure (GD9-12) resulted in increased NE where late gestation (GD 17-20) exposure resulted in decreased NE levels.

Reygner et al., 2016. This study examined the effects of chlorpyrifos on lipid and glucose metabolism, insulin and leptin, gut microbiota composition and short-chain fatty acids (SCFA) production in the developing rat. Pregnant Wistar females were treated with chlorpyrifos at 0 (rapeseed oil), 1, and 3.5 mg/kg/d with or without inulin from GD 1 through PND 21. At PND 21, male pups were weaned and then treated with the same dosing regimen as the dams. There were no effects on dams for body weight, food or water consumption or cholinergic signs. Males at both doses showed increased body weights at birth but body weights and body weight gain were comparable to control (1.0 mg/kg/d) or decreased (3.5 mg/kg/d) at PND 60. Insulin receptor β was decreased and hyperinsulinemia was increased at 1.0 mg/kg/d, while at 3.5 mg/kg/d, males showed decreased insulin and increased hyperglycemia. Both doses showed effects on gut microbiota. The authors conclude that chlorpyrifos may alter body weights, insulin receptors (at the low dose), and induce hyperglycemia and hyperinsulinemia at or above doses associated with ChE inhibition. Leptin levels were not affected and effects did not last into adulthood.

II.N.2.c. Adult Animal Studies on Metabolism and Obesity

Meggs and Brewer, 2007. This study investigated effects of low doses of chlorpyrifos on parameters of weight gain after four months of treatment. Female Long-Evans rats (10/dose) were treated by s.c. injection for four months at 0 (DMSO + saline) and 5.0 mg/kg/d. Animals were examined for cholinergic signs and were weighed at baseline, 2, 3 and 4 months. Body weights increased significantly at 2, 3 and 4 months. Significantly increased perinephric fat pads were measured at termination. Liver weights were slightly increased. Pre-differentiated fat cells were treated with chlorpyrifos in vitro at 0.008 µg/ml or 10 µl DMSO and there was no effect on normal cell growth. There was fat accumulation but no increase in number of cells or increased cell growth. There were, however increases in cell death compared to control.

Wang et al., 2009. The metabolic profiles of serum were examined after chlorpyrifos treatment in adult (M/F; 6-8 week old) Wistar rats to evaluate their metabolic status. The profiles are indicators of metabolite (low molecular weight), proteins (high molecular weight) and lipoprotein particles (supramolecular weight) levels that are detected by ^{3}H-nuclear magnetic resonance (^{3}H-NMR). Rats were treated at 1.30, 3.26, and 8.15 mg/kg/day chlorpyrifos (M) or 1.08, 2.70, and 6.75 mg/kg/d (F) by gavage (corn oil vehicle) for 90 days. Results indicated that serum aminotransferase (ALT) and total bilirubin levels from rats treated at the high dose increased by 29 and 35%, respectively in the absence of histopathology at any dose. Metabolic profiles showed that males and females had similar changes at the mid and high doses compared to controls. Chlorpyrifos treatment led to disruption of key ketone-metabolizing enzymes in the liver mitochondria and protein metabolism in the liver was also affected, as shown by a high level of glycoprotein. The authors conclude that chlorpyrifos at doses of 2.7 mg/kg/d and greater can disrupt energy production and fatty acid metabolism in the absence of histopathology in the liver and blood chemistry changes.

Peris-Sampedro et al., 2015b. A strain of mice expressing the human apolipoprotein E3 (apoE3) genetic isoform were used in this study to examine the association between this gene and obesity and related metabolic disorders. ApoE3, from the apoE gene, is a protein that combines with lipids (e.g., cholesterol and other fats) to form lipoproteins which can then be transported through the blood. Male TR apoE3 mice (homozygous for the human E3 allele) and C57BL/6N male mice were treated with CPF in diet at 0 or 2.0 mg/kg/d for 8 weeks. Animals were checked for cholinergic signs twice per week, bodyweights and food and water consumption were measured and plasma ChE activity was assayed. Metabolic biomarkers (total cholesterol, triglycerides, albumin, creatinine, aspartate (AST) and alanine (ALT) transaminases) and insulin sensitivity were measured. Insulin sensitivity was estimated by measuring fasting plasma insulin and calculating an insulin resistance score (homeostatic model assessment for insulin resistance [IR]; HOMA-IR = (fasting insulin x fasting glucose)/22.5). Plasma leptin, total ghrelin (orexigenic [appetite-stimulating] hormone from stomach or brain) and acyl ghrelin (circulating form of ghrelin) levels were quantified. Plasma ChE was inhibited by 68% after 8 weeks in both CPF-treated genotypes. In chlorpyrifos-treated mice, food intake (both genotypes) and body weights were statistically significantly increased weeks 4 through 8 in apoE3 mice as compared with week 8 only in C57BL/6N mice. Plasma metabolic biomarkers (cholesterol and triglycerides) in chlorpyrifos-treated apoE3 mice were increased.

Fang et al., 2018. This study was performed to investigate the effect of chlorpyrifos on the microbiota in relation to potential risk factors for obesity, diabetes, and neurotoxicity. Adult male Wistar rats (8 weeks old) were fed a normal fat (NF) or high fat (HF) diet and were gavaged with either 0 (DMSO in saline + tween), 0.3 (normal fat-low, NF-L or high fat-low, HF-L), or 3.0 mg/kg/d chlorpyrifos (normal fat-high, NF-H or high fat-high, HF-H; 6/dose) for 9 weeks. Plasma glucose was decreased in the normal fat/low dose animals, but only at 60 and 90 minutes, not at 9 weeks. There were no significant effects on total triglycerides, total cholesterol, HDL-C or LDL-C. However, animals in the high fat diet group showed increased triglycerides. Animals receiving doses of 0.3 mg/kg/d chlorpyrifos showed decreases in peptides compared to controls, although the effect was not shown at the higher dose.

II.O. Recent Advances in Chlorpyrifos PBPK Modeling

A recent study by Zurlinden and Reisfeld (2018) proposed a method to use a health-based end point in conjunction with the existing validated PBPK-PD model to estimate a benchmark dose for chlorpyrifos. The authors first generated an exposure space database by running the PBPK-PD model for a total of 10,000 Monte-Carlo sampling draws based on four exposure parameters (exposure route, dose, exposure periodicity, and exposure duration) in a 30-day subchronic exposure setting. They then selected an in vivo rat study (Yan *et al.*, 2012) as a validation dataset to connect an internal dose metric (peak brain chlorpyrifos concentration) to a health-based end point (a cognitive deficit in spatial learning from Yan study). The PBPK model was then used to derive corresponding peak brain chlorpyrifos concentrations for different exposure doses (0, 1, 5, 10 mg/kg). A mathematic dose-response model-Emax (Hill) equation was used to describe the relationship between predicted peak brain chlorpyrifos concentration and observed fractional cognitive deficit. The peak brain chlorpyrifos concentration giving rise to a 15% cognitive deficit was selected as the PoD benchmark dose, which corresponded to a peak brain chlorpyrifos

concentration of 8.82 x 10^{-6} µM. This concentration is approximately 19.6-fold lower than the peak brain chlorpyrifos associated with 20% RBC AChE inhibition and 54.8-fold lower than the peak brain chlorpyrifos associated with 10% brain AChE inhibition (Zurlinden and Reisfeld, 2018). This dose-response model was subsequently used to generate a corresponding fractional cognitive deficit data point for each simulated exposure scenario based on the predicted peak brain chlorpyrifos concentration from the exposure space database generated at the beginning for both rats and humans. The authors then used a mathematic equation to relate the cognitive deficit end point to predicted plasma chlorpyrifos concentration in rats.

Additional explanation of the author's findings are beyond the scope of this assessment, however HHA concludes that successful application of this novel approach requires a validated interspecies PBPK-PD model for internal dose prediction, a critical dose metric to serve as the internal dose across species, and a quantifiable behavioral outcome observed in dose-response in animals. Some of the main limitations of the study include: 1) behavioral endpoints in the rats are not adequately correlated to cognitive deficits in humans; 2) use of a validation dataset based on chlorpyrifos dose levels that can be overtly toxic; and, 3) the assumption that chlorpyrifos parent is the penultimate toxicant associated with neurobehavioral deficits. Additionally, HHA is concerned with several mathematical errors found in the publication including in the formula used to convert enzyme availability to inhibition and in calculations for percent of enzyme inhibition corresponding to the threshold cognitive deficit. As such, HHA will reevaluate this approach as appropriate when new data become available.

III. HAZARD IDENTIFICATION

III.A. Introduction

Critical points of departure (PoD) for chlorpyrifos were established from animal studies reporting DNT effects at dose levels that are generally considered lower than those necessary for RBC AChE inhibition. As defined by US EPA (2012a), a point of departure is the dose-response point that marks the starting point for low-dose extrapolation, and the PoD generally corresponds to a selected estimated low-level of response. For the in vivo animal DNT studies used in this risk assessment, the primary exposure route is oral.

III.B. Acute and Short-Term Toxicity

III.B.1. Oral Toxicity

The human epidemiological studies that showed association between chlorpyrifos exposure during gestation and impacts on human growth and development could not be used to establish critical PoDs for DNT because exposure-effect relationships were not completely elucidated and because of concerns with analytical methodologies used for quantifying exposure. While many DNT studies in animals were available for chlorpyrifos, the focus for this assessment was on studies that reported neurodevelopmental effects occurring at doses lower than those causing AChE inhibition. The toxicity studies that were considered for establishing critical neurodevelopmental PoDs are listed in Table 11.

Five recently published studies reported developmental toxicity in rodents at doses causing minimal or no brain AChE inhibition. Four of these studies used rats and one study was conducted in mice. In every case, exposure was by the oral route (three by gavage, two through the diet). Two studies employed both gestational and lactational exposure through the dams (a total of 35 doses, 14 consecutive daily doses during pregnancy and 21 doses during lactation). Two studies employed direct pup exposure for either one or seven days starting at PND 10. Neurodevelopmental responses in offspring were tested either in in young pups (PNDs 21-25) or in adults (60-90 days). Three studies reported increased motor or total activity, two studies showed altered anxiety levels (decreased or increased), and one study detected impaired spatial learning. LOELs for the observed neurodevelopmental effects were 0.1-0.5 mg/kg/day. In four of the studies, the LOEL was the lowest tested dose. Applying an uncertainty factor of 10 to those LOELs would result in an estimated no effect level (ENEL) for DNT of 0.01-0.05 mg/kg/day. One study included a NOEL dose based on increased anxiety and motor activity in rats that were exposed in utero with chlorpyrifos for 6 days (Silva *et al.*, 2017). Only one study concurrently measured AChE activity, setting the LOEL for brain AChE inhibition at 1.0 mg/kg/day (Carr *et al.*, 2017).

A registrant-submitted DNT study measured brain, RBC, and plasma ChE in addition to neurodevelopmental outcomes (Hoberman, 1998). This study employed both gestational and lactational exposure through the dams (a total of 26 doses, 15 consecutive daily doses during pregnancy and 11 doses during lactation). RBC AChE inhibition was the most sensitive endpoint in this study, with a $BMDL_{10}$ / BMD_{10} of 0.03 / 0.06 mg/kg/day. HHA set the developmental LOEL at 1 mg/kg/day for reduced cortex and hippocampal dimensions in PND 66-71 females. This LOEL was 10 fold higher than the LOEL for DNT reported in the published studies.

In conclusion, new findings from published animal studies indicated that the developing nervous system is sensitive to low doses of chlorpyrifos that are not expected to inhibit brain or RBC AChE activities. Based on the five studies in Table 11, the collective LOEL for neurodevelopmental effects including in cognition, motor control, and behavior in rats and mice is 0.1 mg/kg/day. A NOEL of 0.01 mg/kg/day was established by Silva et al., (2017) based on increased anxiety and motor activity in rat pups. This NOEL is supported by the ENELs of 0.05-0.01 mg/kg/day estimated from the DNT LOELs of 0.5-0.1 by applying a 10 fold UF. The exposure duration in the 5 published studies varied from 1 to 35 days. Therefore, the NOEL of 0.01 mg/kg/day could be applicable to acute and repeated exposures to chlorpyrifos in infants, children, and females of childbearing age. A more conservative approach when considering developmental effects is that they occur as the result of a single acute exposure, rather than ongoing or cumulative exposures. Therefore in the remainder of this assessment, HHA uses the assumption that chlorpyrifos-mediated developmental toxicity may result from a single exposure equivalent to 0.01 mg/kg/day.

Table 11. Selected Developmental Neurotoxicity Studies in Rats and Mice

Species, Dosing Period, Doses (mg/kg/day)	Cholinesterase Inhibition				Developmental Neurotoxicity		Study
	Time tested	LOEL NOEL			Effects	LOEL NOEL	
		Plasma	RBC	Brain			
Gestation and postnatal exposure							
Rat Gavage GD 6-LD 11 0.3, 1.0, 5.0	Dam LD 22	0.3 --	0.06[a] **0.03**[b]	0.65[a] **0.54**[b]	Reduced parietal cortex and hippocampal dimensions in PND 66-71 females	1.0 --	Hoberman, 1998
Rat Diet GD 7- PND 21 0.1, 0.3, 1.0	Not tested	--	--	--	Decreased spatial learning in 2-3 month old males	0.1 --	Gómez-Giménez et al., 2017
Rat Diet GD 7- PND 21 0.1, 0.3, 1.0	Not tested	--	--	--	Increased spontaneous motor activity in 2-3 month old males and females	0.1 --	Gómez-Giménez et al., 2018
Gestation-only exposure							
Rat Gavage GD 14-20 0.01, 0.1, 1.0, 10	Not tested	--	--	--	Increased anxiety and locomotor activity in PND21 males	0.1 **0.01**	Silva et al., 2017
Postnatal- only exposure							
Rat Gavage PND 10-16 0.5, 0.75 & 1.0	Pups PND 16	--	--	1.0 **0.75**	Decreased anxiety in PND25 males and females	0.5 --	Carr et al., 2017
Mouse Gavage PND 10 0.1, 1.0, 5.0	Pups PND 10	--	--	5.0 --	Increased total activity in PND 60 males	0.1 --	Lee et al., 2015

[a] BMD_{10} –BMD analysis in US EPA, 2011
[b] $BMDL_{10}$ –BMD analysis in US EPA, 2011
Abbreviations: LOEL, lowest observed effect level; NOEL, no observed effect level ; GD, gestation day; LD, lactation day; PND, postnatal day
Red text denotes the study NOEL, if available

III.B.2. Dermal and Inhalation Toxicity

Studies were not available to establish dermal and inhalation PODs for developmental neurotoxicity. Therefore, the acute oral PoD of 0.01 mg/kg/day was used to evaluate acute dermal and inhalation exposures using route-to-route extrapolation.

IV. EXPOSURE ASSESSMENT

The following is an update to the exposure assessment from the December 2017 Draft TAC Evaluation.

IV.A. Introduction

Spray Drift Exposure Estimates

Exposure associated with chlorpyrifos spray drift near an application site was evaluated for four population subgroups: infants, children 1-2 years old, children 6-12 years old, and females of childbearing age (13-49 years old). These groups were chosen because of the assumed susceptibility to chlorpyrifos-related developmental neurotoxicity, the critical endpoint used in this risk assessment. The standard operating procedure (SOP) assumed that the turf contact duration of exposure for infants, children 1-2 years old, children 6-12 years old, and females of childbearing age (13-49 years old) near the application sites would be 1.5 hours and inhalation exposure duration is 1 hour. The US EPA Residential SOP (Addenda 1: Consideration of Spray Drift) argues that children 1-2 years old exhibit the highest exposure potential to pesticides on contaminated lawn from spray drift because of dermal contact and different mouthing activities such as hand-to-mouth, object-to-mouth, and incidental soil ingestion (US EPA, 2012a). As such, US EPA determined that children 1 – 2 years old represent the most appropriate index childhood life stage for most individual SOPs. However, for completeness and following suggestions made at the January and March 2018 SRP hearings, HHA has expanded this exposure assessment to include infants (< 1 year old) as well as children 6 – 12 years old.

Values for all assumptions necessary in estimating exposures are not available for all four age groups, so several replacement values were used. Exposure routes for children 1 – 2 years old are well characterized (including for incidental oral exposure). The same is not true for infants between 6 – 12 months. As such, this exposure assessment used the transfer coefficient for children 1 – 2 years old combined with the infant body weight and breathing weight assumptions to estimate dermal exposure for infants. The same held true for mouthing activities, where the assumptions for children 1 – 2 years old are better characterized than they are for infants. Therefore, the dermal exposure and incidental oral exposure from hand-to-mouth and object-to-mouth activities derived for infants may be overestimates of the actual exposure values. To estimate exposures for children 6-12 years old, it was necessary to use the adult transfer coefficient for dermal contact, although age specific body weight and breathing rates were available to complete the exposure characterization. Incidental oral exposure from hand-to-mouth or object-to-mouth activities was not estimated for children 6 – 12 years old or for females of childbearing age (13 – 49 years old) because that type of activity have a very low occurrence in those age groups (Xue *et al.*, 2010).

Aerial Applications

Single application exposure estimates via horizontal deposition (in mg/kg/day) and inhalation as both inhalation exposure (in mg/kg/day) and 1 hour time-weighted average air concentrations (in mg/m^3) of chlorpyrifos were considered for four subpopulations: infants, children 1-2 years old, children 6-12 years old, and females of childbearing age (13-49 years old) and three application rates for two types of aircraft: fixed-wing (AT802A airplane) and rotary (Bell 205 helicopters). Increases in chlorpyrifos application rates resulted in a corresponding increase in the exposure estimates.

The standard practice at DPR is to calculate exposure estimates based on single application scenarios. Exposure estimates for multiple or simultaneous applications are considered the purview of risk mitigation and management and, as such, are not included in the exposure

analysis of a specific pesticide. For this exposure assessment, and later for evaluating the margins of exposure, HHA used a fixed wing aerial application of chlorpyrifos with 2 gallons/acre finished spray volume and 2 lbs/acre application rate as its standard exposure scenario. This reflects the most common aircraft used for aerial applications in California, as well as the most common and reasonable "worst case" scenario for application rates and volumes. The reader will find calculated estimates for dermal, oral, and inhalation doses and air concentrations for several other application rates and volumes for fixed wing aircraft in Tables 12 – 17, below. A complete listing of exposure estimates for all aircraft types, application rates and volumes, and application types can be found in Appendix 2 herein. Additional background information about the assumptions used in the exposure analysis can be found in the December 2017 Draft TAC Evaluation.

Ground-Based Applications

Horizontal deposition exposure estimates (in mg/kg/day) of chlorpyrifos were evaluated for the same four population subgroups at four application rates, up to the labeled maximum rate, with two ground-based application methods: ground boom and airblast. For ground boom, horizontal deposition estimates were derived using two swath percentiles: 50^{th} and 90^{th}. Horizontal deposition exposure estimates of chlorpyrifos after ground boom or airblast application showed that exposure increases with increasing application rates. The higher horizontal deposition exposure estimates of the high-boom compared with the low-boom is consistent with the difference in the spray release height above the target between high- and low-boom (50 and 20 inches above the target, respectively). All other factors held constant, horizontal deposition increases as a function of boom height above the target. The higher near-field horizontal deposition exposure estimates shown by orchard airblast compared to ground boom are consistent with the much finer droplet spectrum of the airblast sprayer application method and the upward direction by the airblast sprayer of fine spray into the orchard canopy. See Appendix 2 for complete results of the exposure estimations for ground boom and airblast.

IV.B. Spray Drift Exposure Assessment Approach

For assessing the short-term exposure due to off-site movement of chlorpyrifos, this exposure assessment adopted the method of US EPA (Dawson et al., 2012); that is, spray drift modeling coupled with post-application assessment of dermal and inhalation exposures. For the spray drift modeling, two computer models were employed: AgDRIFT (spray drift regression model version 2.0.05) for ground boom and orchard airblast applications; and, AGDISP (AGricultural DISPersal near-wake Lagrangian model version 8.28) for aerial applications (Barry, 2015). For the post-application assessment, the US EPA SOP for residential exposure assessment was followed (US EPA, 2013). Spray drift air concentrations were modeled from 25 to 2608 feet. The range of modeled distances was chosen because a buffer zone of 25 feet is required for aerial application of chlorpyrifos and 2608 feet is the computational limit of the model.

Technical description of these models and exposure estimation methods have been detailed elsewhere (Teske *et al.*, 2002a; Teske *et al.*, 2002b; Barry, 2015). Both AgDRIFT and AGDISP models were used to estimate off-site horizontal deposition of chlorpyrifos at different distances downwind. Scenarios and input parameter values were chosen to represent the reasonable worst case application conditions so that spray drift is not underestimated for the application scenarios

assessed. AGDISP was used to estimate horizontal deposition and 1 hour time-weighted average air concentrations (mg/m^3) of chlorpyrifos at vertical heights of 1.7 ft, and 5 ft. The vertical heights of 1.7 ft represents the breathing zones of infants and children 1-2 years old, 5 ft represents the breathing zones of children 6-12 years old, and females 13-49 years old. The aerial application exposure scenarios evaluated in this exposure assessment used the estimated air concentrations for each specific scenario. For airblast and ground boom, horizontal deposition was estimated with AgDRIFT but the AGDISP model was used to produce surrogate air concentrations using a default aerial application (fixed wing AT802A aircraft with a finished spray volume of 2 gal/acre) and the specific application rates for each airblast and ground boom scenario evaluated in this exposure assessment. This choice of surrogate air concentrations has been previously used by US EPA to characterize inhalation exposures due to spray drift associated with orchard airblast and ground boom applications (Dawson *et al.*, 2012); US EPA 2012b). The AGDISP model is a mass conserving model and provides an air concentration calculated based on the airborne mass passing through a flux plane at specific distances. The mass includes all active ingredient material still airborne when the spray drift cloud passes a particular flux plane. HHA assumes that all mass in the air is 100% available and absorbed.

IV.C. Spray Drift Exposure Estimates

A complete analysis of spray drift exposure estimates along with margins of exposure can be found in Appendix 2 of this document.

IV.C.1. Aerial Applications

Tables 12 and 13 show primary spray drift exposure estimates for fixed wing aircraft applying 2 lbs chlorpyrifos /acre application rate and 2 gallons/acre (GPA) finished spray. Exposures due to contact with chlorpyrifos deposited on turf and to inhalation of chlorpyrifos residues in the air are shown. The Infant age group shows the highest exposure due to the smallest body weight and the highest breathing rate. In addition, exposures for all age groups increase with increasing application rate but within a single application rate exposures decrease with increasing distance downwind from the application. A full set of exposure estimates for additional aerial application exposure scenarios can be found in Appendix 2. These additional scenarios include fixed wing and helicopter application methods in addition to application rates of 1, 2, and 2.3 lbs chlorpyrifos /acre application rates at 2 GPA and 15 GPA finished spray volumes. A full discussion on aerial application scenario development methods and primary spray drift can be found in (Barry, 2017).

IV.C.2.Ground-Based Applications

Tables 14 and 15 show primary spray drift exposure due to horizontal deposition onto turf from a dormant apple orchard airblast application at 2 lbs chlorpyrifos/acre application rate. In addition, primary spray drift exposure due to inhalation of chlorpyrifos residues in air is estimated using surrogate air concentrations from the default aerial scenario of fixed wing aircraft applying 2 lbs chlorpyrifos /acre application rate and 2 gallons/acre (GPA) finished spray. Exposure estimates were developed for two types of orchards (dormant apple and sparse orchard) and 4 application rates (1, 2, 4, and 6 lbs chlorpyrifos /acre). The full set of orchard airblast application exposure

estimates can be found in Appendix 2. Discussion on orchard airblast application method scenario development and primary spray drift can be found in Barry, 2017.

Table 16 and 17 show primary spray drift exposure due to horizontal deposition onto turf from a ground boom high boom application. In addition, primary spray drift exposure due to inhalation of chlorpyrifos residues in air is estimated using surrogate air concentrations from the default aerial scenario of fixed wing aircraft applying 2 lbs chlorpyrifos /acre application rate and 2 gallons/acre (GPA) finished spray. For ground boom spray drift deposition estimates were derived for two boom heights (low and high), 4 application rates (1, 2, 4, and 6 lbs chlorpyrifos /acre), and two statistical percentiles (50^{th} and 90^{th}). The full set ground boom application exposure estimates can be found in in Appendix 2. Discussion on ground boom application method scenario development and primary spray drift can be found in Barry, 2017.

For both orchard airblast and ground boom, the infant age group shows the highest exposure due to the smallest body weight and the highest breathing rate. In addition, exposures for all age groups increase with increasing application rate, but within a single application rate exposures decrease with increasing distance downwind from the application.

Table 12. Dermal, Oral, Inhalation Doses, and Inhalation Concentration for Infants and Children 1-2 years old at Various Distances Downwind from the Fields Treated with Chlorpyrifos by AT802A Fixed Wing Aircraft at 2 gallons/acre[a] Finished Spray Volume and 2 lb/acre Chlorpyrifos Application Rate

Age group	Downwind Distance (feet)	Dermal 9.6% Absorption[e] (mg/kg/day)	Hand-to-Mouth (mg/kg/day)	Object-to-Mouth (mg/kg/day)	Soil Ingestion (mg/kg/day)	Inhalation (mg/kg/day)	Inhalation 1-hr TWA Air Concentration (mg/m^3)
Infants[b]	25[d]	0.009937	0.002153	0.000066	0.000016	0.001212	0.0493
	50	0.007792	0.001689	0.000052	0.000013	0.001074	0.0437
	100	0.005205	0.001128	0.000035	0.000008	0.000860	0.0350
	250	0.002605	0.000565	0.000017	0.000004	0.000583	0.0237
	500	0.001418	0.000307	0.000009	0.000002	0.000376	0.0153
	1000	0.000557	0.000121	0.000004	0.000001	0.000177	0.0072
	1320	0.000327	0.000071	0.000002	0.000001	0.000121	0.0049
	2608	0.000061	0.000013	0.000000	0.000000	0.000040	0.0016
Children 1-2 years old[c]	25	0.00581	0.00126	0.000039	0.000009	0.001085	0.0493
	50	0.00456	0.00099	0.000030	0.000007	0.000961	0.0437
	100	0.00304	0.00066	0.000020	0.000005	0.000770	0.0350
	250	0.00152	0.00033	0.000010	0.000002	0.000521	0.0237
	500	0.00083	0.00018	0.000006	0.000001	0.000337	0.0153
	1000	0.00033	0.00007	0.000002	0.000001	0.000158	0.0072
	1320	0.00019	0.00004	0.000001	0.000000	0.000108	0.0049
	2608	0.00004	0.00001	0.000000	0.000000	0.000036	0.0016

[a] Minimum spray volume as specified on some chlorpyrifos product labels for the aerial application.
[b] Infants: Transfer Coefficient (cm^2/hr) = 49000 (US EPA, 2012a); body weight = 7.6 kg; normalized daily average breathing rate = (0.59 m^3/kg/day)/24 hr= 0.025 m^3/kg/hr; breathing height = 1.7 ft (Andrews and Patterson, 2000; See Appendix 4)
[c] Children 1-2 years old: Transfer Coefficient (cm^2/hr) = 49000 (US EPA, 2012a); body weight = 13 kg; normalized daily average breathing rate = (0.52 m^3/kg/day)/24 hr = 0.022 m^3/kg/hr; breathing height = 1.7 ft (Andrews and Patterson, 2000; See Appendix 4)
[d] Buffer zone of 25 feet is required for aerial application of chlorpyrifos.
[e] Data derived chlorpyrifos dermal absorption rate (Thongsinthusak, 1991).

Table 13. Dermal, Oral, Inhalation Doses, and Inhalation Concentration for Children 6-12 years old and Females 13- 49 years old at Various Distances Downwind from the Fields Treated with Chlorpyrifos by AT802A Fixed Wing Aircraft at 2 lb/acre Application Rate and 2 gallons/acre[a] Finished Spray Volume

Age group	Downwind Distance (feet)	Dermal 9.6% Absorption[e] (mg/kg/day)	Inhalation (mg/kg/day)	Inhalation 1-hr TWA Air Concentration (mg/m^3)
Children 6-12 years old[b]	25[d]	0.010670	0.000587	0.0367
	50	0.008367	0.000512	0.0320
	100	0.005589	0.000414	0.0259
	250	0.002798	0.000278	0.0174
	500	0.001522	0.000178	0.0111
	1000	0.000599	0.000083	0.0052
	1320	0.000351	0.000058	0.0036
	2608	0.000065	0.000019	0.0012
Females 13-49 years old[c]	25	0.003864	0.000440	0.0367
	50	0.003030	0.000384	0.0320
	100	0.002024	0.000311	0.0259
	250	0.001013	0.000209	0.0174
	500	0.000551	0.000133	0.0111
	1000	0.000217	0.000062	0.0052
	1320	0.000127	0.000043	0.0036
	2608	0.000024	0.000014	0.0012

[a] Minimum spray volume as specified on some chlorpyrifos product labels for the aerial application.
[b] Children 6-12 years old: Transfer Coefficient (cm^2/hr) = 180000 (US EPA, 2012a); body weight = 26 kg; normalized daily average breathing rate = (0.38 m^3/kg/day)/24 hr = 0.016 m^3/kg/hr; breathing height = 5 ft (Andrews and Patterson, 2000; See Appendix 4)
[c] Females 13-49 years old: Transfer Coefficient (cm^2/hr) = 180000 (US EPA, 2012a); body weight = 71.8 kg; normalized daily average breathing rate = (0.28 m^3/kg/day)/24 hr = 0.012 m^3/kg/hr; breathing height = 5 ft (Andrews and Patterson, 2000; See Appendix 4)
[d] Buffer zone of 25 feet is required for aerial application of chlorpyrifos.
[e] Data derived chlorpyrifos dermal absorption rate (Thongsinthusak, 1991).

Table 14. Dermal, Oral, Inhalation Doses, and Inhalation Concentration for Infants and Children 1-2 years old at Various Distances Downwind from the Fields Treated with Chlorpyrifos by Dormant Apple Orchard Airblast 2 lb/acre Application Rate and Surrogate Air Concentrations using Wing Aircraft at 2 gallons/acre[a] Finished Spray Volume and 2 lb/acre Application Rate

Age group	Downwind Distance (feet)	Dermal 9.6% Absorption[e] (mg/kg/day)	Hand-to-Mouth (mg/kg/day)	Object-to-Mouth (mg/kg/day)	Soil Ingestion (mg/kg/day)	Inhalation (mg/kg/day)	Inhalation 1-hr TWA Air Concentration (mg/m^3)
Infants[b]	25[d]	0.003354	0.000727	0.000022	0.000005	0.001212	0.0493
	50	0.001276	0.000277	0.000008	0.000002	0.001074	0.0437
	100	0.000356	0.000077	0.000002	0.000001	0.000860	0.0350
	250	0.000048	0.000010	0.000000	0.000000	0.000583	0.0237
	500	0.000008	0.000002	0.000000	0.000000	0.000376	0.0153
	1000	0.000002	0.000000	0.000000	0.000000	0.000177	0.0072
	1320	0.000001	0.000000	0.000000	0.000000	0.000120	0.0049
	2608	0.000000	0.000000	0.000000	0.000000	0.000039	0.0016
Children 1-2 years old[c]	25	0.001961	0.000425	0.000013	0.000003	0.001085	0.0493
	50	0.000746	0.000162	0.000005	0.000001	0.000961	0.0437
	100	0.000208	0.000045	0.000001	0.000000	0.000770	0.0350
	250	0.000028	0.000006	0.000000	0.000000	0.000521	0.0237
	500	0.000005	0.000001	0.000000	0.000000	0.000337	0.0153
	1000	0.000001	0.000000	0.000000	0.000000	0.000158	0.0072
	1320	0.000000	0.000000	0.000000	0.000000	0.000108	0.0049
	2608	0.000000	0.000000	0.000000	0.000000	0.000036	0.0016

[a] Minimum spray volume as specified on some chlorpyrifos product labels for the aerial application.
[b] Infants: Transfer Coefficient (cm^2/hr) = 49000 (US EPA, 2012a); body weight = 7.6 kg; normalized daily average breathing rate = (0.59 m^3/kg/day)/24 hr = 0.025 m^3/kg/hr; breathing height = 1.7 ft (Andrews and Patterson, 2000; See Appendix 4)
[c] Children 1-2 years old: Transfer Coefficient (cm^2/hr) = 49000 (US EPA, 2012a); body weight = 13 kg; normalized daily average breathing rate = (0.52 m^3/kg/day)/24 hr = 0.022 m^3/kg/hr; breathing height = 1.7 ft (Andrews and Patterson, 2000; See Appendix 4)
[d] Buffer zone of 25 feet is required for aerial application of chlorpyrifos.
[e] Data derived chlorpyrifos dermal absorption rate (Thongsinthusak, 1991).

Table 15. Dermal, Oral, Inhalation Doses, and Inhalation Concentration for Children 6-12 years old and Females 13- 49 years old at Various Distances Downwind from the Fields Treated with Chlorpyrifos by Dormant Apple Orchard Airblast 2 lb/acre Application Rate and Surrogate Air Concentrations using Wing Aircraft at 2 lb/acre Application Rate and 2 gallons/acre[a] Finished Spray Volume

Age group	Downwind Distance (feet)	Dermal 9.6% Absorption[e] (mg/kg/day)	Inhalation (mg/kg/day)	Inhalation 1-hr TWA Air Concentration (mg/m^3)
Children 6-12 years old[b]	25[d]	0.003601	0.000587	0.0367
	50	0.001370	0.000512	0.0320
	100	0.000382	0.000414	0.0259
	250	0.000051	0.000278	0.0174
	500	0.000009	0.000178	0.0111
	1000	0.000002	0.000083	0.0052
	1320	0.000001	0.000058	0.0036
	2608	0.000000	0.000019	0.0012
Females 13-49 years old[c]	25	0.001304	0.000440	0.0367
	50	0.000496	0.000384	0.0320
	100	0.000138	0.000311	0.0259
	250	0.000019	0.000209	0.0174
	500	0.000003	0.000133	0.0111
	1000	0.000001	0.000062	0.0052
	1320	0.000000	0.000043	0.0036
	2608	0.000000	0.000014	0.0012

[a] Minimum spray volume as specified on some chlorpyrifos product labels for the aerial application.
[b] Children 6-12 years old: Transfer Coefficient (cm^2/hr) = 180000 (US EPA, 2012a); body weight = 26 kg; normalized daily average breathing rate = (0.38 m^3/kg/day)/24 hr = 0.016 m^3/kg/hr; breathing height = 5 ft (Andrews and Patterson, 2000; See Appendix 4)
[c] Females 13-49 years old: Transfer Coefficient (cm^2/hr) = 180000 (US EPA, 2012a); body weight = 71.8 kg; normalized daily average breathing rate = (0.28 m^3/kg/day)/24 hr = 0.012 m^3/kg/hr; breathing height = 5 ft (Andrews and Patterson, 2000; See Appendix 4)
[d] Buffer zone of 25 feet is required for aerial application of chlorpyrifos.
[e] Data derived chlorpyrifos dermal absorption rate (Thongsinthusak, 1991).

Table 16. Dermal, Oral, Inhalation Doses, and Inhalation Concentration for Infants and Children 1-2 years old at Various Distances Downwind from the Fields Treated with Chlorpyrifos by Ground Boom High Boom at 2 lb/acre Application Rate and Surrogate Air Concentrations using Wing Aircraft at 2 lb/acre Application Rate and 2 gallons/acre[a] Finished Spray Volume

Age group	Downwind Distance (feet)	Dermal 9.6% Absorption[e] (mg/kg/day)	Hand-to-Mouth (mg/kg/day)	Object-to-Mouth (mg/kg/day)	Soil Ingestion (mg/kg/day)	Inhalation (mg/kg/day)	Inhalation 1-hr TWA Air Concentration (mg/m^3)
Infants[b]	25[d]	0.000576	0.000125	0.000004	0.000001	0.001212	0.0493
	50	0.000382	0.000083	0.000003	0.000001	0.001074	0.0437
	100	0.000224	0.000049	0.000001	0.000000	0.000860	0.0350
	250	0.000103	0.000022	0.000001	0.000000	0.000583	0.0237
	500	0.000044	0.000010	0.000000	0.000000	0.000376	0.0153
	1000	0.000013	0.000003	0.000000	0.000000	0.000177	0.0072
	1320	0.000007	0.000002	0.000000	0.000000	0.000120	0.0049
	2608	0.000001	0.000000	0.000000	0.000000	0.000039	0.0016
Children 1-2 years old[c]	25	0.000337	0.000073	0.000002	0.000001	0.001085	0.0493
	50	0.000223	0.000048	0.000001	0.000000	0.000961	0.0437
	100	0.000131	0.000028	0.000001	0.000000	0.000770	0.0350
	250	0.000060	0.000013	0.000000	0.000000	0.000521	0.0237
	500	0.000026	0.000006	0.000000	0.000000	0.000337	0.0153
	1000	0.000008	0.000002	0.000000	0.000000	0.000158	0.0072
	1320	0.000004	0.000001	0.000000	0.000000	0.000108	0.0049
	2608	0.000001	0.000000	0.000000	0.000000	0.000036	0.0016

[a] Minimum spray volume as specified on some chlorpyrifos product labels for the aerial application.
[b] Infants: Transfer Coefficient (cm^2/hr) = 49000 (US EPA, 2012a); body weight = 7.6 kg; normalized daily average breathing rate = (0.59 m^3/kg/day)/24 hr = 0.025 m^3/kg/hr; breathing height = 1.7 ft (Andrews and Patterson, 2000; See Appendix 4)
[c] Children 1-2 years old: Transfer Coefficient (cm^2/hr) = 49000 (US EPA, 2012a); body weight = 13 kg; normalized daily average breathing rate = (0.52 m^3/kg/day)/24hr = 0.022 m^3/kg/hr; breathing height = 1.7 ft (Andrews and Patterson, 2000; See Appendix 4)
[d] Buffer zone of 25 feet is required for aerial application of chlorpyrifos.
[e] Data derived chlorpyrifos dermal absorption rate (Thongsinthusak, 1991).

Table 17. Dermal, Oral, Inhalation Doses, and Inhalation Concentration for Children 6-12 years old and Females 13- 49 years old at Various Distances Downwind from the Fields Treated with Chlorpyrifos by Ground Boom High Boom at 2 lb/acre Application Rate and Surrogate Air Concentrations using Wing Aircraft at 2 lb/acre Application Rate and 2 gallons/acre[a] Finished Spray Volume

Age group	Downwind Distance (feet)	Dermal 9.6% Absorption[e] (mg/kg/day)	Inhalation (mg/kg/day)	Inhalation 1-hr TWA Air Concentration (mg/m^3)
Children 6-12 years old[b]	25[d]	0.000618	0.000587	0.0367
	50	0.000410	0.000512	0.0320
	100	0.000241	0.000414	0.0259
	250	0.000111	0.000278	0.0174
	500	0.000047	0.000178	0.0111
	1000	0.000014	0.000083	0.0052
	1320	0.000008	0.000058	0.0036
	2608	0.000001	0.000019	0.0012
Females 13-49 years old[c]	25	0.000224	0.000440	0.0367
	50	0.000148	0.000384	0.0320
	100	0.000087	0.000311	0.0259
	250	0.000040	0.000209	0.0174
	500	0.000017	0.000133	0.0111
	1000	0.000005	0.000062	0.0052
	1320	0.000003	0.000043	0.0036
	2608	0.000000	0.000014	0.0012

[a] Minimum spray volume as specified on some chlorpyrifos product labels for the aerial application.
[b] Children 6-12 years old: Transfer Coefficient (cm^2/hr) = 180000 (US EPA, 2012a); body weight = 26 kg; normalized daily average breathing rate = (0.38 m^3/kg/day)/24 hr = 0.016 m^3/kg/hr; breathing height = 5 ft (Andrews and Patterson, 2000; See Appendix 4)
[c] Females 13-49 years old: Transfer Coefficient (cm^2/hr) = 180000 (US EPA, 2012a); body weight = 71.8 kg; normalized daily average breathing rate = (0.28 m^3/kg/day)/24 hr = 0.012 m^3/kg/hr; breathing height = 5 ft (Andrews and Patterson, 2000; See Appendix 4)
[d] Buffer zone of 25 feet is required for aerial application of chlorpyrifos.
[e] Data derived chlorpyrifos dermal absorption rate (Thongsinthusak, 1991.

IV.D. Secondary Drift Exposure Estimates

As suggested at the January and March 2018 SRP hearings, HHA re-evaluated the potential influence of secondary drift on total exposure risk to chlorpyrifos. The most recent 5 years of data within the DPR Air Monitoring Network (AMN) (http://www.cdpr.ca.gov/docs/emon/airinit/air_network_results.htm) were used to assess the potential for exposure due to secondary drift (re-volatilization). Air concentrations and 24-hr inhalation exposures are shown in Table 18. The 24-hr TWA air samples collected by the AMN include both primary drift from applications in the area close to a particular sampler in addition to any secondary drift from those applications. Thus, the results shown in Table 18 are likely overestimates of secondary drift exposures. Because of the very small influence of secondary drift on the total exposure estimates as calculated herein, the influence of secondary drift was excluded from further exposure analysis calculations. Note that both the modeled air concentrations (above) and the monitored air concentrations (Table 18) are denoted in units of mg chlorpyrifos/m^3 air.

Table 18. Air Monitoring Network Highest Ambient Air Concentrations over the Most Recent Five Years and the 24-hr Inhalation Exposure Based on those Air Concentrations for Infants, Children 1-2 years old, Children 6-12 years old, and Females 13-49 years old

	Summary of Samples				24-hr Inhalation Exposure (mg/kg/day)			
Year	Total number of samples	Detections	Quantified	Highest 24-hr concentration (mg/m^3)	Infant[a]	Child 1-2 years old[b]	Child 6-12 years old[c]	Females 13-49 years old[d]
2016	156	21	3	0.0000521	0.000031	0.000027	0.000020	0.000015
2015	155	45	6	0.0000778	0.000046	0.000040	0.000030	0.000022
2014	157	38	4	0.0003379	0.000199	0.000176	0.000128	0.000095
2013	159	52	5	0.0004225	0.000249	0.000220	0.000161	0.000118
2012	156	44	3	0.0001309	0.000077	0.000068	0.000050	0.000037

[a] Infants: body weight = 7.6 kg; normalized daily average breathing rate = (0.59 m^3/kg/day)
[b] Children 1-2 years old: body weight = 13 kg; normalized daily average breathing rate = (0.52 m^3/kg/day)
[c] Children 6-12 years old: body weight = 26 kg; normalized daily average breathing rate = (0.38 m^3/kg/day)
[d] Females 13-49 years old: body weight = 71.8 kg; normalized daily average breathing rate = (0.28 m^3/kg/day)
For references see Andrews and Patterson, 2000; Appendix 4.

IV.E. Exposure from House Dust

As suggested at the January and March 2018 SRP hearings, HHA re-evaluated potential exposure to chlorpyrifos through contaminated house dust. Inhalation of airborne material, dermal contact with contaminated surfaces, and non-dietary oral ingestion (e.g., pica) are all potential exposures of chlorpyrifos associated with spray drift following pesticide applications. Young children tend to spend more time on the floor and have more incidental oral exposure (hand-to-mouth, object-to-mouth) than older children or adults (Xue *et al.*, 2010; Dawson *et al.*, 2012). Therefore it is important to assess potential chlorpyrifos exposures that may occur via

incidental ingestion of contaminated indoor dust, especially in young children in agricultural families or who live in agricultural areas (Quiros-Alcala *et al.*, 2011; Gunier *et al.*, 2016). Prior to the restrictions of indoor use, house dust may have been contaminated with chlorpyrifos residues derived from the indoor applications (e.g., in home insect control) (Lewis *et al.*, 2001) or from "take-home" exposure from occupational settings (Fenske *et al.*, 2013; Gibbs *et al.*, 2017; Smith *et al.*, 2017). In 2000, US EPA heavily restricted indoor chlorpyrifos use, leaving only roach baits in child resistant packaging registered for indoor use.[3] Therefore, sources outside of the home can now be assumed to be the sole contributors to chlorpyrifos residues in house dust.

Chlorpyrifos concentrations were measured in house dust samples collected from farmworker residences in the Salinas Valley, CA in 1999 and 2002 (Bradman *et al.*, 2007; Harnly *et al.*, 2009). In the studies by Bradman et al. (2007) and Harnly et al. (2009), a high-volume surface sampler with a cyclone was used to collect dust samples then analyzed by GC-MS for residual chlorpyrifos concentration. The authors reported that maximum concentrations in house dust decreased from 9810 ng/g dust in 1999 to 1200 ng/g dust in 2002. Because these household dust samples were collected from homes of farmworkers within the same agricultural area, the substantial decrease in the maximum house dust concentrations over this time period suggests that indoor use may have been the major source of chlorpyrifos in contaminated house dust. After the restrictions of home use, outdoor sources such as "take-home" by farmworkers became the dominant source of chlorpyrifos in the home. Likewise, Quiros-Alcala and colleagues compared 15 farmworker residences in the same area of Salinas, CA as the 1999-2002 study and found that chlorpyrifos concentrations in house dust were approximately 40% lower in 2006 (Quiros-Alcala *et al.*, 2011).

In another study, Gunier et al. (2016) collected house dust samples from 434 California homes of study subjects enrolled in either the Northern California Childhood Leukemia Study (n=413) or the Fresno-County based Agricultural Pesticide Study (n=21). Of the samples collected, 388 (89%) had detectable chlorpyrifos concentrations above the limit of detection (3 ng/g dust), with a 90^{th} percentile of 220 ng/g dust and the geometric mean of 34 (±5) ng/g dust across the study period of 2001 – 2006 (Gunier et al., 2016). Chlorpyrifos concentrations in house dust decreased an average of 31% per year ($p < 0.0001$) across all samples. When homes in the Central Valley were analyzed separately, the decrease was not as large (27% decrease), but still highly significant (Gunier et al., 2016). Dust samples collected from the Fresno County homes from 2003 – 2005 did not shown the same year over year decrease; the authors postulate that this is due to a fairly steady agricultural use of chlorpyrifos during the same time. These study values are plotted against the pounds of chlorpyrifos used in California from 1999 to 2006 (Figure 1). Based on this analysis, indoor chlorpyrifos concentrations have continued a precipitous decline from 1999 to 2006 in California, although the pounds of chlorpyrifos applied agriculturally do

[3] Chlorpyrifos; Cancellation Order. A Notice by the Environmental Protection Agency on 12/06/2000. Federal Register, https://www.federalregister.gov/documents/2000/12/06/00-30917/chlorpyrifos-cancellation-order

not mirror the same decline. This supports several authors' supposition that the major reason for reductions in indoor concentrations comes from the federal cancellation of indoor use.

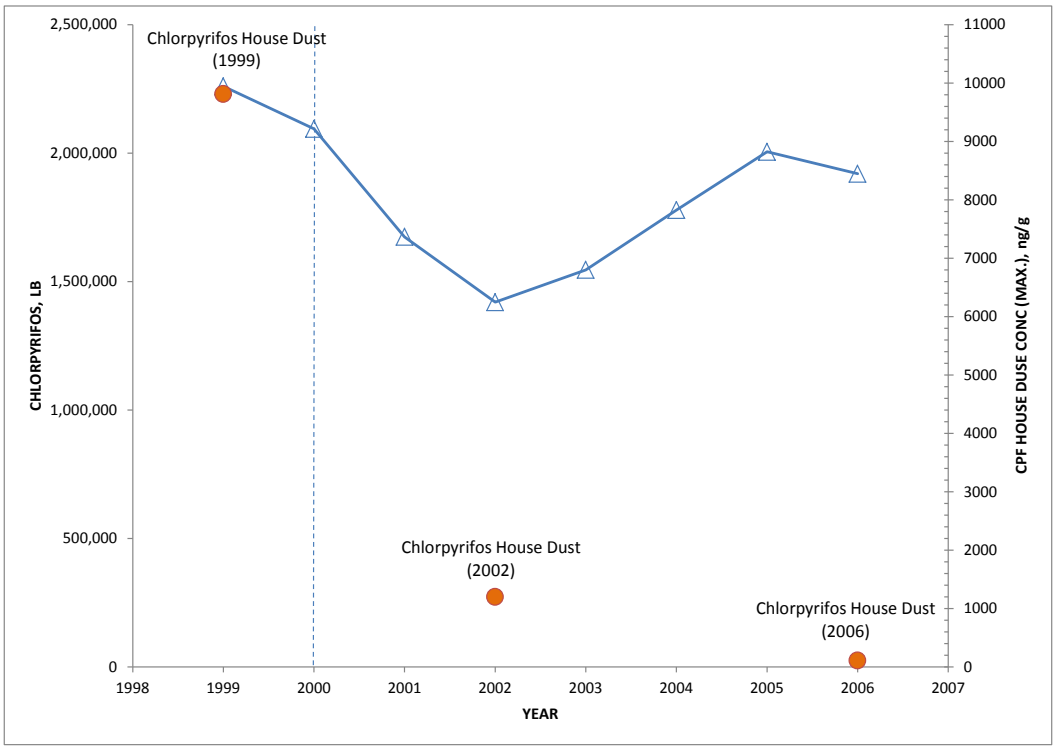

Figure 1. Pounds of chlorpyrifos applied in California from 1999 to 2006 and maximum concentrations of chlorpyrifos measured in house dust samples collected from inside California homes in 1999, 2002, and 2006

Studies have shown that chlorpyrifos concentrations in house dust are higher in farmworker homes than non-farmworker homes in both California (Quiros-Alcala *et al.*, 2011) and Washington states (Gibbs *et al.*, 2017; Smith *et al.*, 2017). Accordingly, assessing the house dust exposure in farmworker homes with a life stage that has the highest estimate of soil ingestion rate (i.e., children <2 years old) would constitute a reasonable "worst case" estimate of chlorpyrifos exposure in children. To evaluate children's exposure to chlorpyrifos via house dust, this assessment employs house dust concentrations of chlorpyrifos collected in California after the indoor use cancellation. Combining the highest measured concentration (i.e., 1200 ng/g) from Bradman et al., (2007) with a daily dust ingestion rate for children 0 - 2 years old (95th%-ile; (OEHHA, 2012), and assuming an infant body (i.e., <1 yr old) weight of 7.6 kg (DPR, 2000), and 100% oral absorption, a short term absorbed daily dose (STADD) can be estimated as 0.048 µg/kg/day. If using the maximum chlorpyrifos house dust concentration measured in 2006 (Gunier *et al.*, 2016) instead, the estimated STADD is 0.0044 µg/kg/day. With these updated exposure estimates from house dust, it is clear that chlorpyrifos exposure via house dust would only contribute minimally to the overall or aggregate exposure estimates. Therefore, house dust was removed from further exposure analysis calculations.

IV.F. Dietary Exposure (Food and Drinking Water)

The following is a new analysis of the risk from food and drinking water and has been completely updated from the December 2017 Draft TAC Evaluation. For complete background information and methodology on how HHA conducts dietary exposure assessment, the reader is directed to Section IV.B. Dietary Exposure (Food and Drinking Water), in the December 2017 Draft TAC Evaluation.

Briefly, HHA utilized the 2014 US EPA food-only exposure estimates to evaluate the risk from chlorpyrifos exposure from food (US EPA, 2014). HHA conducted an independent drinking water exposure assessment employing residue data from refined, surface, and ground water in California.

US EPA estimated dietary (food only) acute and steady-state exposures for infants (< 1 year old), children (1-2 years old), children (6-12 years old), and females (13-49 years old). The dietary analyses were conducted with Dietary Exposure Evaluation Model (DEEM) and Calendex software with the Food Commodity Intake Database (FCID). The food consumption data in the software was based on the 2003-2008 from the U.S. Department of Agriculture's (USDA's) National Health and Nutrition Examination Survey, What We Eat in America, (NHANES/ WWEIA). Dietary consumption data were combined with residue data from the US Department of Agriculture Pesticide Data Program (through 2012) to estimate exposures based on probabilistic analysis. The steady-state exposure estimates were determined using the Calendex-FCID program, which utilizes the same consumption database and residue data as DEEM-FCID. The steady-state or steady-state exposures were derived for 21-day period. The exposure values are shown in the Tables 19 and 20. Children 1-2 year old were identified to receive the highest exposure from food at the 99.9th percentile in both acute and steady-state exposure scenarios.

Table 19. Acute Dietary Exposure for Chlorpyrifos

Population Subgroup	Dietary Exposure (mg/kg/d)		
	95th Percentile	99th Percentile	99.9th Percentile
All Infants < 1 year old	0.000050	0.000088	0.000273
Children 1-2 years old	0.000082	0.000143	0.000423
Children 6-12 years old	0.000040	0.000072	0.000189
Females 13-49 years old	0.000021	0.000041	0.000150

Table 20. Steady-State Dietary Exposure for Chlorpyrifos

Population Subgroup	Dietary Exposure (mg/kg/d)		
	70th Percentile	95th Percentile	99.9th Percentile
All Infants < 1 year old	0.000020	0.000045	0.000186
Children 1-2 years old	0.000038	0.000072	0.000242
Children 6-12 years old	0.000019	0.000039	0.000128
Females 13-49 years old	0.000009	0.000018	0.000075

The drinking water exposure was calculated based on residues from PDP and DPR surface and ground water programs. The probabilistic exposures at the 95th, 99th and 99.9th percentiles are shown in Table 21. Infants were identified as the most highly exposed subpopulation.

Table 21. Acute Drinking Water Exposure for Chlorpyrifos

Drinking Water Exposure (mg/kg/day)			
2001-2013 PDP Residue Data			
Population Subgroup	95th	99th	99.9th
All Infants (< 1 year old)	0.000004	0.000064	0.000113
Children 1-2 years old	0.000002	0.000026	0.000060
Children 6-12 years old	0.000002	0.000016	0.000038
Females 13-49 years old	0.000001	0.000018	0.000038
2005-2014 DPR Surface Water Residue Data			
Population Subgroup	95th	99th	99.9th
All Infants (< 1 year old)	0.000008	0.000051	0.000439
Children 1-2 years old	0.000004	0.000024	0.000186
Children 6-12 years old	0.000002	0.000015	0.000115
Females 13-49 years old	0.000002	0.000016	0.000125
2004-2013 DPR Ground Water Residue Data			
Population Subgroup	95th	99th	99.9th
All Infants (< 1 year old)	0.000019	0.000133	0.000233
Children 1-2 years old	0.000013	0.000057	0.000121
Children 6-12 years old	0.000008	0.000032	0.000079
Females 13-49 years old	0.000009	0.000038	0.000077

The PDP data indicate that chlorpyrifos residues are frequently detected on crops that lack chlorpyrifos tolerances. This could result from illegal applications on these crops, drift from applications to nearby fields, or soil residues remaining from applications to an earlier crop previously grown in the same field. From 2008 to 2012, PDP detected illegal chlorpyrifos residues on catfish, cilantro, cherry tomatoes, green onions, spinach, and five other crops. From 2015 to 2017, DPR's California Pesticide Residue Monitoring Program (CPRMP) had 280 detections of chlorpyrifos from more than 3602 samples tested. A total of 58 detections were illegal (Table 22). Litchi, cactus, longan, and oriental pear had frequent illegal chlorpyrifos detections. Most of these were imported produce. US EPA sets the legal limit (tolerance) for the amount of pesticide residues allowed in food. Over the years, DPR's residue monitoring program has detected illegal chlorpyrifos residues on various commodities, most or all of which were imported (Table 22) for residues detected from 2015-2017). Neither DPR nor US EPA assesses the health implications of illegal residues on agricultural commodities in their dietary exposure assessments, which are restricted to analyzing the health implications of legal residues. However, DPR's Enforcement Branch enforces US EPA tolerances under the CPRMP, which collects domestic and imported produce samples throughout the channels of trade, including wholesale and retail outlets, distribution centers, and farmers markets. These samples are analyzed for pesticide residues at laboratories run by the California Department of Food and Agriculture (CDFA). When a pesticide residue is determined to be illegal by virtue of (a) its occurrence on a commodity for which there is no established tolerance; or (b) its level exceeding the established tolerance, HHA conducts a special dietary exposure assessment to determine if an acute health risk exists from consumption of that lot. The results are then communicated to the Enforcement Branch, which has the authority to remove affected produce from channels of trade.

Table 22. Commodities Sampled by DPR's Pesticide Residue Monitoring Program Containing Chlorpyrifos Residues from 2015 to 2017

Commodities with CPF detections	Total no. samples tested	Samples with detections	No. illegal samples[a]
LITCHI NUTS	26	16	16
PEAR, ASIAN (ORIENTAL PEAR)	69	18	10
PRICKLYPEAR CACTUS PADS	94	9	9
PRICKLYPEAR (CACTUS PEAR)	40	11	8
LONGAN (LONGAN FRUIT)	31	7	7
TOMATILLO	187	5	2
BEANS (GREEN, STRING)	203	2	1
CHAYOTE (CHRISTOPHENES)	114	2	1
TARO (DASHEEN) (ROOT CROP) (WETLAND, UPLAND, ETC.)	17	1	1
RAMBUTAN	5	1	1
PASSION FRUIT (TAMARILLO, PURPLE GRANADILLA)	4	1	1
ARROWHEAD (SAGITTARIA SPP.)	1	1	1
ORANGE (ALL OR UNSPEC)	270	65	0
PEPPERS (FRUITING VEGETABLE), (BELL,CHILI, ETC.)	545	50	0
TANGERINE (MANDARIN, SATSUMA, MURCOTT, ETC.)	213	33	0
BANANA	155	22	0
LEMON	80	8	0
LIME (MEXICAN LIME, PERSIAN, ETC.)	143	5	0
RADISH TOPS	29	4	0
NECTARINE	246	3	0
ASPARAGUS (SPEARS, FERNS, ETC.)	168	3	0
TURNIPS (ALL OR UNSPEC)	17	3	0
KALE	327	2	0
KIWI FRUIT	106	2	0
PEA, SNOW (SUGAR PEA)	125	1	0
CHINESE RADISH/DAIKON (LOBOK, JAPANESE RADISH)	118	1	0
BOK CHOY (WONG BOK)	109	1	0
PINEAPPLE (FRESH MKT. PINEAPPLE)	90	1	0
RADISH	58	1	0
PLANTAIN	12	1	0
Totals	**3602**	**280**	**58**

[a] Illegal samples are those in which a pesticide residue occurs on a commodity for which there is no established tolerance; or its level exceeding the established tolerance; data from the California Pesticide Residue Monitoring Program.

Following suggestions received during the 2018 SRP hearings, HHA also looked more closely at the risk to children of consuming almond milk as a potential means of exposure to chlorpyrifos. The following acute exposure and risk calculation for chlorpyrifos residue in almond milk is based on consumption data in 1-12 year old children in NHANES (2011-2014). Because almond milk is not an agricultural crop, HHA had to research manufacturing based recipes to determine the equivalent quantity of almonds in almond milk. The most popular commercial brand of almond milk contains 2% almonds. Using the maximum individual consumption rate of almond

milk for children 1 - 12 years old, the assumption that almond milk is comprised of 2% almonds, and the 99th percentile chlorpyrifos residue measured in whole almonds, the acute exposure level is estimated at 0.000076 mg/kg/day. This is compared to the maximum individual consumption rate of the same age group for whole almonds which is 0.0038 mg/kg/day. The calculated residue levels in almond milk ranging from 0.000036 to 0.000956 ppm (for 99th percentile to the highest residue respectively) are less than the tolerance for almonds, and are below the CDFA and PDP detection limits of 0.01 ppm and 0.001 ppm, respectively. Using the DNT PoD, consumption of whole almonds would be below the MOE and considered a potential health risk, while the consumption of almond milk because of its small percentage of almonds would not.

V. RISK CHARACTERIZATION

V.A. Introduction

For this risk assessment, the risk for threshold effects is expressed as a margin of exposure (MOE). The MOE is the ratio of the critical NOEL or PoD to the estimated human exposure level.

V.B. Risk Characterization using PoDs for Developmental Neurotoxicity

The neurodevelopmental effects analyzed in this assessment can be grouped as changes in cognition, motor control, or behavior. None of the in vivo animal studies used inhalation or dermal exposure routes; only oral dosing was used (diet or gavage). A NOEL of 0.01 mg/kg/day was observed in only one DNT study and based on increased anxiety and motor activity in PND21 male rat pups at 0.1 mg/kg/day (Silva *et al.*, 2017). The NOEL of 0.01 mg/kg/day is similar to an estimated no effect level (ENEL) if the LOELs from the other four studies had been divided by a default uncertainty factor of 10 (summarized in Table 11). Therefore, the critical NOEL selected to evaluate the risk for potential neurodevelopmental effects from acute exposures to chlorpyrifos was 0.01 mg/kg/day based on the NOEL from Silva et al. (2017) and the ENELs from the other DNT studies (Lee *et al.*, 2015; Carr *et al.*, 2017; Gomez-Gimenez *et al.*, 2017; Gomez-Gimenez *et al.*, 2018).

Table 23. Critical NOELs for Developmental Neurotoxicity used for the Risk Characterization of Chlorpyrifos

Route	PoD[a]	RfD[b] or RfC
Uncertainty Factors (UF)		**10 inter 10 intra 1 DNT**
Acute Oral [mg/kg/day] Infants Children 1-2 Children 6-12 Females 13-49	0.01	0.0001
Acute Dermal [mg/kg/day][c] Infants Children 1-2 Children 6-12 Females 13-49	0.104	0.001
Acute Inhalation [mg/m^3][c] Infants Children 1-2 Children 6-12 Females 13-49	 0.405 0.459 0.624 0.862	 0.004 0.005 0.006 0.009

[a] Point of Departure (PoD): The critical acute oral PoD for CPF is a NOEL (No-Observed Effect Level) for developmental neurotoxicity based on changes in cognition, motor control and behavior in rats and mice (Lee et al, 2015, Silva et al, 2017, Carr et al, 2017, Gómez-Giménez, 2017, 2018).

[b] Reference Dose (RfD) or Reference Concentration (RfC): RfDs and RfCs are derived by dividing the appropriate PoD by the product of all uncertainty factors (UF).

[c] Route to route extrapolation:

Dermal: Route specific dermal PoD: oral PoD in animals (mg/kg/day) / dermal absorption in human (9.6% ; Thongsinthusak, 1991).

Inhalation: Route specific inhalation PoD: oral dose mg/kg/day / [Breathing Rate (BR) m^3/hr/Body Weight (BW) kg]; Oral PoD=0.01 mg/kg/day; Infants BR=0.188 m^3/h BW= 7.6 kg; Children 1-2 yrs BR=0.283 m^3/h BW=13 kg; Children 6-12 yrs BR= 0.417 m^3/h, BW=26 kg; Females 13-49 yrs BR=0.833 m^3/h, BW 71.8 kg (derived from Andrews and Patterson (2000) assuming 24-hr breathing rates of 0.59, 0.52, 0.38 and 0.28 m^3/kg/24 hr for infants, children 1-2 yr, children 6-12 yr and females 13-49 yr, respectively.) [See Appendix 4.]

V.C. Spray-Drift Bystander (Non-Occupational/Residential)

Risks for bystanders were calculated for exposures from a standard scenario using fixed wing aerial application of chlorpyrifos with 2 gallons/acre finished spray volume and 2 lbs/acre application rate. This scenario reflects the most common aircraft used for aerial applications in California, as well as the most common and a reasonable “worst case” estimate. The exposure assessment calculations for all other scenarios, application methods, and application rates and volumes can be found in Appendix 2. Only acute exposure to spray drift from single aerial applications of chlorpyrifos was evaluated in this assessment, as is the standard practice for DPR exposure estimates calculations. Exposure estimates for multiple or simultaneous applications are considered the purview of risk mitigation and management and, as such, are not included in the exposure analysis of a specific pesticide. Air concentrations were modeled to the computation downwind distance limit, e.g., 2608 feet downwind from an application. HHA

acknowledges that it is possible to detect concentrations of chlorpyrifos in ambient air at levels at or above the analytical limit of quantitation at distances farther downwind from an application than ½ mile (2640 feet).

Route-to-route extrapolation was performed by converting the external dermal and inhalation doses to internal doses. This was necessary since inhalation specific NOELs were not available to evaluate the potential risk for neurodevelopmental effects from inhalation of chlorpyrifos (required for the evaluation of toxic air contaminants). For calculating inhalation doses, the estimated air concentrations (found in Section IV earlier in this document) were multiplied by a default breathing rate of 0.59, 0.52 and 0.38 m^3/kg/day (or 0.025, 0.022 and 0.016 m^3/kg/hr) for infants, children 1-2 years old and children 6-12 years old, respectively, or by 0.28 m^3/kg/day (or 0.0112 m^3/kg/hr) for females 13-49 years old (Andrews and Patterson, 2000, Appendix 4). A default absorption rate of 100% was assumed for inhalation exposure. For dermal doses, the external dermal dose was multiplied by a dermal absorption factor of 9.6% based on evaluation of the available chlorpyrifos dermal absorption studies (Thongsinthusak, 1991).

When inhalation, dermal, and incidental oral exposures from spray drift were evaluated using the DNT NOEL of 0.01 mg/kg/day, the combined drift MOEs were less than 100 at ≤ 1320 feet from the treated field for all of the evaluated populations, indicating a health concern. The dermal MOEs were lower than the inhalation MOEs at each distance. As a result, the combined drift MOEs were lower than the dermal MOEs. The combined drift MOEs were greater than 100 only at 2608 feet for all four sensitive population subgroups, indicating that at this distance and at distances further downwind, there is not a health concern for aggregate exposure from inhalation or deposition from spray drift. The margins of exposure are summarized in Table 24, below. Values below the target of 100 are denoted with red shading.

Table 24. Margins of Exposure using the Developmental Neurotoxicity NOEL for Infants, Children, and Females of Childbearing Age at Various Distances Downwind from the Fields Treated with Chlorpyrifos by Fixed Wing Aircraft at 2 gallons/acre Spray Volume and 2 lb/acre Application Rate

Age group	Downwind Distance (ft)	Margins of Exposure [a]			
		Dermal	Combined Incidental Oral	Inhalation	Combined Drift
Infants < 1 year	25	1	4	8	<1
	50	1	6	9	<1
	100	2	9	12	1
	250	4	17	17	3
	500	7	31	27	5
	1000	18	80	56	12
	1320	31	136	83	19
	2608	165	734	250	87
Children 1-2 years	25	2	8	9	1
	50	2	10	10	2
	100	3	15	13	3
	250	7	29	19	5
	500	12	54	30	9
	1000	31	136	63	21
	1320	52	232	92	33
	2608	282	1255	279	140
Children 6-12 years	25	1	--	17	1
	50	1	--	20	1
	100	2	--	24	2
	250	4	--	36	3
	500	7	--	56	6
	1000	17	--	120	15
	1320	28	--	174	24
	2608	154	--	521	119
Females 13-49 years	25	3	--	23	2
	50	3	--	26	3
	100	5	--	32	4
	250	10	--	48	8
	500	18	--	75	15
	1000	46	--	160	36
	1320	79	--	231	59
	2608	424	--	694	263

[a] Risks were calculated as a margin of exposure (MOE) for infants, children, youths, and females of childbearing age. A target MOE of 100 was selected to be protective of human health (10x for interspecies sensitivity, 10x for intraspecies variability). DNT NOEL = 0.01 mg/kg/day based on changes in cognition, motor control and behavior in rats and mice (Lee et al, 2015, Silva et al, 2017, Carr et al, 2017, Gómez-Giménez , 2017, 2018).
Red shading indicates MOEs that are below the target of 100, thus indicating a potential health concern.

V.D. Dietary Exposure

The acute dietary and drinking water MOEs were calculated using the oral NOEL of 0.01 mg/kg/day for developmental neurotoxicity in rats and mice. The DNT effects were seen after single day exposure or repeated treatments. Therefore the same NOEL is applicable to repeated (steady-state) exposures to chlorpyrifos. The acute dietary MOEs ranged from 122 to 476 at the 95th percentile, from 70 to 244 at the 99th percentile and from 24 to 67 at the 99.9th percentile. The steady state MOEs ranged from 139 to 556 (95th percentile) and from 41to 133 (99.9th percentile). Children 1-2 yrs were identified as the most highly exposed population. In a probabilistic dietary analysis, both DPR and US EPA present the risk using dietary exposures at the 99.9th percentile. The margins of exposure for acute and steady-state dietary exposures are summarized in Table 25 and for drinking water in Table 26. Values below the target of 100 in both tables are denoted with red shading.

Table 25. Acute and Steady-State Dietary (food only) Exposure and Margins of Exposure for Chlorpyrifos

ACUTE DIETARY EXPOSURE[a]				
Population Subgroup	**aPoD[b] (mg/kg)**	**MOE[c]**		
		95th Percentile	**99th Percentile**	**99.9th Percentile**
All Infants: < 1 yr	0.01	200	114	37
Children: 1-2 yrs	0.01	122	70	24
Children: 6-12 yrs	0.01	250	139	53
Females: 13-49 yrs	0.01	476	244	67
STEADY-STATE DIETARY EXPOSURE[a]				
Population Subgroup	**ssPoD[b] (mg/kg)**	**MOE[c]**		
		70th Percentile	**95th Percentile**	**99.9th Percentile**
All Infants: < 1 yr	0.01	500	222	54
Children: 1-2 yrs	0.01	263	139	41
Children: 6-12 yrs	0.01	526	256	78
Females: 13-49 yrs	0.01	1111	556	133

[a] Exposures are from the US EPA dietary exposure assessment to support registration review (US EPA, 2014b)
[b] aPoD = acute point of departure
[c] Margin of Exposure (MOE) = PoD ÷ Dietary Exposure. Target MOE is 100 for every population.
Red shading indicates MOEs that are below the target of 100.

For drinking water exposure, the risks were calculated using the NOEL of 0.01 mg/kg/day for DNT effects and probabilistic exposures based on residues from PDP and DPR surface and ground water programs (Table 26). The exposure levels at the 99.9th percentile, the MOEs were higher for PDP (88 – 263) and lower for surface water (23 – 87). Infants were identified as the most highly exposed population from drinking water.

Table 26. Acute Margins of Exposure for Chlorpyrifos in Drinking Water

Population Subgroup	2001-2013 PDP Residue Data			2005-2014 Surface Water Residue Data		
	MOE			MOE		
	95th	99th	99.9th	95th	99th	99.9th
All Infants (< 1 year old)	2500	156	88	1250	196	23
Children 1-2 years old	5000	385	167	2500	417	54
Children 6-12 years old	5000	625	263	5000	625	80
Females 13-49 years old	10000	556	263	5000	667	87

Population Subgroup	2004-2013 Ground Water Residue Data		
	MOE		
	95th	99th	99.9th
All Infants (< 1 year old)	526	75	43
Children 1-2 years old	769	175	83
Children 6-12 years old	1250	313	127
Females 13-49 years old	1111	263	130

V.D. Aggregate Exposure (Spray Drift, Dietary, and Drinking Water)

Combined spray drift exposures estimates at 2608 feet for dermal, incidental oral, and inhalation routes were combined with the 99.9th percentile exposures from dietary and drinking water for chlorpyrifos. At 2608 feet from a field treated with chlorpyrifos, the combined spray drift MOEs for three of the sensitive population subgroups were equal to or greater than the target of 100. However, when dietary and drinking water exposures were added in, the aggregate MOEs for these combined routes and sources of exposure were below all the target of 100 (Table 27).

Table 27. Margins of Exposure using the DNT NOEL for Combined Spray Drift, Dietary and Drinking Water Exposure at 2608 ft from Field Treated with Chlorpyrifos for Infants, Children and Females of Childbearing Age

Population Subgroup	Margin of Exposure[a]			
	Diet Only[b]	Drinking Water Only[b,c]	Combined Spray Drift[d]	Combined Spray Drift, Diet and Drinking Water[e]
All Infants < 1 year	37	23	87	12
Children 1-2 years	24	54	140	15
Children 6-12 years	53	87	119	26
Females 13-49 years	67	80	263	32

Abbreviations: DNT = Developmental Neurotoxicity, NOEL = No-Observed-Effect Level.

[a] Margin of Exposure (MOE) = NOEL / Exposure ; DNT NOEL = 0.01 mg/kg/day based on changes in cognition, motor control and behavior in rats and mice (Lee et al, 2015, Silva et al, 2017, Carr et al, 2017, Gómez-Giménez, 2017, 2018)

[b] Dietary exposure estimate at the 99.9th percentile was used in the MOE calculation

[c] Drinking water exposure estimate based on the 99.9th percentile from DPR surface water monitoring was used in the MOE calculation

[d] Combined Spray Drift MOE is the MOE for the combined dermal, incidental oral and inhalation exposure from spray drift at 2608 ft from the treated field which is the only distance where MOEs were greater than 100 for all routes (see Table 24).

[e] Combined MOE = DNT NOEL (0.01) / (Diet + Drinking Water + Combined Spray Drift) Exposure.

Red shading indicates MOEs that are below the target of 100.

VI. RISK APPRAISAL

VI.A. Introduction

This final TAC evaluation of chlorpyrifos explores in greater depth the potential for adverse impacts on the developing nervous system. The December 2017 draft recognized developmental neurotoxicity as likely to be biologically significant, but did not carry the analysis further, opting instead to apply a 10-fold uncertainty factor to the cholinesterase-based endpoints to account for potential neurodevelopmental effects. Original selection of RBC AChE inhibition as the critical toxicity endpoint was intended to protect human populations from impacts on other neurological endpoints that are not as easily measured. However, collective results from epidemiology and animal toxicity studies indicate that chlorpyrifos may cause neurodevelopmental and neurobehavioral effects in the absence AChE inhibition.

This risk assessment evaluated the dietary, spray drift, and aggregate risks that accompany exposure to chlorpyrifos. Every risk assessment has inherent limitations with the application of existing data to estimate potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for chlorpyrifos are delineated in the following discussion.

VI.B. Uncertainties Associated with the Toxicology and Hazard Identification

Comprehensive analysis of the developmental toxicity database has now allowed HHA to set a critical acute NOEL for neurodevelopmental effects at 0.01 mg/kg based on a limited number of studies in rats and mice. Most relevant in this regard is the observation of increased anxiogenic behavior in the elevated plus-maze test and motor activity in PND 21 rat pups exposed in utero (GD 14-20) to a maternal gavage dose of 0.1 mg/kg/day (gestation only) (Silva et al., 2017). Similar motor effects were observed by Gómez-Giménez et al. (2017) in PND 60-90 rat pups and by Lee et al. (2015) in PND 60 mouse pups both at doses of 0.1 mg/kg. However in Gómez-Giménez et al., (2017), the treatment period was gestational and postnatal, while the treatment period in Lee et al. (2015) was postnatal only. In both cases, observations were made long after cessation of dosing, suggesting that the neurotoxic impacts of early life exposure have the potential to be long-lasting. In addition, Gómez-Giménez et al. (2017) observed cognitive deficits at 0.1 mg/kg/d and Carr et al. (2017) showed decreased anxiety in PND 25 male rats following gavage exposure to 0.5 mg/kg/d on PND 10 – 16.

Because neurodevelopmental observations were made at similar doses by several laboratories, HHA considered the critical NOEL to be reasonably supported. Nonetheless, there were several factors associated with uncertainty in the NOEL designation:

1) One detailed study failed to show cognitive effects in maze testing even at gestational / postnatal doses as high as 5 mg/kg/day (Hoberman, 1998). This was surprising in light of

the observations in later studies of effects at 0.1 mg/kg. Since there are some epidemiology studies showing an association of chlorpyrifos exposure and changes in growth and development, the rodent studies were considered relevant because they yielded qualitative similar responses.

2) Both anxiogenic and anti-anxiogenic responses were observed in the DNT studies, highlighting the possibility that the effects were mutable and possibly toxicologically insignificant. However, HHA notes that the anxiogenic behavior observed by Silva et al. (2017) resulted from gestational exposure, while the anti-anxiogenic behavior observed by Carr et al. (2017) resulted from postnatal exposure. As the developmental status of the very young organism changes with time, the precise staging of chlorpyrifos exposures likely affects the nature of the response.

3) Use of maze-based behaviors as the method for discerning cognitive deficits may not cover the more complex neurological functions in humans. Therefore, its direct relevancy is unknown.

4) Hoberman (1998) observed brain morphometric changes at doses as low as 1 mg/kg/day. Unfortunately, none of the more recent studies reviewed herein attempted such detailed histological or morphometric measurements. It is possible that more contemporary techniques might allow detection of subtle changes in physical parameters.

5) The motor / behavioral data which showing effects at 0.1 mg/kg (and in the case of Silva et al., 2017, a NOEL of 0.01 mg/kg) were not amenable to further analysis because they were presented largely as summary data without reporting individual data, means, or standard deviations. Dose-response relationship not always evident and often missing. Without individual data it is difficult to ascertain the details of what were often subtle effects.

In conclusion, the developmental neurotoxicity database for chlorpyrifos is evolving and currently contains five in vivo animal studies that permit the establishment of a critical oral NOEL. The neurodevelopmental effects in these studies were similar regardless of the exposure window or the duration of the exposure. The most important implication of the five studies is that the threshold for chlorpyrifos-induced neurodevelopmental effects following exposure in early life may be 10-fold lower than the reported threshold of 1 mg/kg/day established for RBC AChE inhibition.

VI.C. Uncertainties Related to Exposure Assessment

This revised exposure assessment evaluated risk to bystanders from spray drift from aerial and ground-based applications of chlorpyrifos and estimated exposures from dermal, inhalation, and incidental oral exposure routes. Inhalation and dermal bystander exposures were evaluated for all four population subgroups. The evaluated exposure scenarios were based on standard operating procedures for lawns and turf post-application, and assumed exposure times near the application site of 1-1.5 hr. In addition, infants and children 1-2 yrs were assumed to receive additional exposure (incidental oral) from spray drift deposition through mouthing activities, such as hand-

to-mouth and object-to-mouth activities, as well as incidental soil ingestion. Several uncertainties exist with the exposure analysis for chlorpyrifos, many of which result from the use of standard default assumptions. A synopsis of these uncertainties follows:

1) For the horizontal deposition exposure calculations, California-specific turf transferable residue (TTR) values obtained from the study by Stafford and Robb (1999) were used. In the same study by these investigators, the mean $TTR_{Day\ 0}$ data ($\mu g/cm^2$) were also obtained from two other states (mean values in parentheses): Indiana (0.09 ± 0.005) and Mississippi (0.146 ± 0.005). Although the value from Mississippi (i.e., the highest value) is not used in the horizontal deposition estimates because California specific data is more appropriate. In addition, this value is comparable to the TTR value obtained in California (0.124 ± 0.004).

2) For acute spray drift exposure estimates, the main uncertainties associated with the computer models used to estimate the exposure to residential bystanders were discussed in the December 2017 Draft TAC Evaluation (DPR, 2017). Those estimates largely depend on the distances from the application site and the model used parameters (wind speed, wind direction, physicochemical properties of chlorpyrifos vapor and aerosol, etc.) that maximized offsite drift estimates.

3) From the revised calculations, it was found that there was minimal contribution to overall exposure from 1) secondary spray drift following the re-volatilization of applied chlorpyrifos and 2) chlorpyrifos-contaminated house dust that was used to calculate the short-term absorbed daily dose. Neither value will alter the combined (inhalation, dermal, and incidental oral) exposures estimates from primary spray drift and deposition. Therefore, these values were removed from the final exposure analysis. The re-analysis of potential exposure from these additional sources was based on the best available and most current data. If new data or analyses become available, HHA will reconsider the contribution of either secondary spray drift or dust exposures to the exposure estimates for chlorpyrifos.

4) Additional uncertainties were associated with use of default physiological parameters, such as body weight and inhalation rates. Uncertainties also accompany the route-to-route extrapolation used in this risk assessment to convert modeled external dermal doses and inhalation concentrations to internal doses.

5) It is standard practice to use default assumptions when estimating exposure through various routes. In some instances this will overestimate actual exposure, such as applying the hand-to-mouth incidental oral exposure estimates for children 1-2 to infants. In some instances using default values may underestimate actual exposure, such as when using average breathing rates for young children who can have higher breathing rates when they are engaged in high intensity physical activity. Default values were not available for all subpopulations for all routes of exposure, such as pregnancy-specific breathing rates and body weight assumptions for children 6-12. Using the same default value for every individual in each age range renders the estimated exposures for the whole age range less representative of specific ages within that range. Some estimates, on the other hand, were specific to chlorpyrifos, such as the 9.6% dermal absorption rate.

VI.C.2. Uncertainties Relation to Dietary and Drinking Water Exposure Assessment

Exposures from diet and drinking water were estimated in the 2017 December Draft TAC Evaluation and the associated uncertainties can be found in the Risk Appraisal section of that document.

VI.D. Uncertainties in the Risk Characterization

VI.D.1. Developmental Neurotoxicity

The target MOE of 100 was considered sufficiently protective of human health. The MOE consisted of 10x for interspecies sensitivity and 10x for intraspecies variability.

VI.D.2. Cholinesterase Inhibition

In the 2017 Draft TAC Evaluation, HHA set a target MOE of 100 (1 for interspecies sensitivity, 10 for intraspecies variability, and 10 for potential neurodevelopmental effects) when exposures were evaluated with the PBPK-PD derived human PoDs for 10% RBC AChE inhibition. Based on suggestions received during the January and March 2018 SRP hearings, and after further evaluation of the PBPK model, the interspecies sensitivity component of the UF was increased 3x to account for PBPK-PD model deficiencies in human inhalation parameters. While a control human study on inhalation exposure was available for the chlorpyrifos model evaluation (Vaccaro *et al.,* 1993), inhalation toxicity data were limited in animals and not available for humans, and therefore not incorporated into the current version of the model (Poet *et al.*, 2017).

VI.E. Evaluation of the Points of Departure and Reference Concentration/Doses for Chlorpyrifos

For this final TAC evaluation of chlorpyrifos, HHA conducted a comprehensive review of animal studies published from 2015 – 2018, focused on the potential for evidence of neurodevelopmental toxicity at low dose levels. Critical PoDs were established from animal studies reporting developmental neurotoxicity at dose levels that are generally considered lower than those necessary for RBC AChE inhibition. A target MOE of 100 was comprised of 10x for interspecies sensitivity and 10x for intraspecies variability. There is no need for an additional UF for neurodevelopmental effects. RfDs and RfCs were calculated by dividing the DNT PoDs by the total UF of 100. These values are shown in Table 28, below. The PoDs for AChE inhibition along with the RfDs and RfCs calculated using both the original total UF of 100 and the revised total UF of 300 are also shown in Table 28 for comparison purposes only. The full analysis of the AChE inhibition based PoDs and MOEs are found in Appendix 3, herein.

Table 28. Points of Departure and Reference Doses or Concentrations used to evaluate the Risk from Exposure to Chlorpyrifos in Selected Population Subgroups for Developmental Neurotoxicity (DNT) and Acetylcholinesterase (AChE) Inhibition

Route	DNT[a]		10% AChE Inhibition	
	PoD[b]	RfD[c] or RfC	PBPK-PD PoD[d]	RfD or RfC (PoD/UF of 300)
Uncertainty Factors (UF)		**10 interspecies 10 intraspecies 1 DNT**		**3 interspecies 10 intraspecies 10 DNT**
Acute Oral [mg/kg/day] Infants Children 1-2 Children 6-12 Females 13-49	0.01	0.0001	 0.600 0.581 0.530 0.469	 0.002 0.002 0.002 0.002
Acute Dermal* [mg/kg/day] Infants Children 1-2 Children 6-12 Females 13-49	0.104	0.001	 NA 134.3 NA 23.6	 NA 0.448 NA 0.079
Acute Inhalation* [mg/m^3] Infants Children 1-2 Children 6-12 Females 13-49	0.405 0.459 0.624 0.862	0.004 0.005 0.006 0.009	NA 2.85 NA 6.15	NA 0.0095 NA 0.0205

[a] DNT, Developmental Neurotoxicity

[b] PoD, Point of Departure (PoD): a starting dose point for low-dose extrapolation. The critical acute oral PoD for chlorpyrifos is NOEL (No-Observed Effect Level) for developmental neurotoxicity in animals based on changes in cognition, motor control and behavior in rats and mice (Lee et al, 2015, Silva et al, 2017, Carr et al, 2017, Gómez-Giménez, 2017, 2018).

[c] RfD, Reference Dose or Reference Concentration (RfC): As defined by US EPA, RfC or RfD is an estimate of the concentration or dose of a substance to which a human populations can be exposed (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime; derived by dividing the appropriate PoD by the product of all uncertainty factors (UF).

[d] The PoDs are Physiologically-Based Pharmacokinetic-Pharmacodynamic (PBPK-PD) model derived human equivalent doses based on 10% inhibition of acetylcholinesterase (AChE) in red blood cells after an acute (single day, 24 hr) or steady-state (21-day) exposure to chlorpyrifos. PBPK-derived PoDs were used in the December 2017 Draft Evaluation of Chlorpyrifos as a Toxic Air Contaminant (Appendix 6) to derive RfDs/RfCs and to calculate risk from exposure to chlorpyrifos.

* Route to route extrapolation:

Dermal: Route specific dermal PoD: oral PoD in animals (mg/kg/day) / dermal absorption in human (9.6%; Thongsinthusak, 1991)

Inhalation: Route specific inhalation PoD: oral dose mg/kg/day / [Breathing Rate (BR) m^3/hr/Body Weight (BW) kg]; Oral PoD=0.01 mg/kg/day; Infants BR=0.188 m^3/h BW= 7.6 kg; Children 1-2 yrs BR=0.283 m^3/h BW=13 kg; Children 6-12 yrs BR= 0.417 m^3/h, BW=26 kg; Females 13-49 yrs BR=0.833 m^3/h, BW 71.8 kg (derived from Andrews and Patterson (2000) assuming 24-hr breathing rates of 0.59, 0.52, 0.38 and 0.28 m^3/kg/24 hr for infants, children 1-2 yr, children 6-12 yr and females 13-49 yr, respectively.) [See Appendix 4.]

NA – Not available for this population

VI.F. Criteria for Evaluating Chlorpyrifos as a Toxic Air Contaminant

For the designation of a pesticide as a TAC, according to the California Code of Regulations, Title 3, Section 6864, for noncancer effects, the threshold levels is 10x below the air concentration which has been determined by the Director of DPR to be protective of human health. The purpose of this assessment is to provide the scientific evidence and evaluation of data that support the designation of chlorpyrifos as a TAC. As such, this evaluation had to assess the following:

- The availability and quality of data on health effects
- The potency, mode of action, and other relevant biological factors
- An estimate of the levels of exposure that may cause or contribute to adverse health effects; and,
- The range of risk to humans resulting from current or anticipated exposure (Food and Agriculture Code § 14023(a)).

A pesticide TAC can be defined as the air concentration, either measured or modeled, that exceeds the reference concentration (RfC) divided by 10. Chlorpyrifos meets the criteria of TAC designation by using either the developmental neurotoxicity endpoint or the AChE inhibition endpoint. If using the acute inhalation RfC for children 1-2 years old based on the DNT endpoint (0.005 mg/m^3; Table 28), chlorpyrifos would be designated a TAC if ambient air concentrations were > 0.0005 mg/m^3. If using the acute inhalation RfC for children 1-2 years old based on the AChE inhibition endpoint (0.0095 mg/m^3; Table 28), chlorpyrifos would be designated a TAC if ambient air concentrations were > 0.00095 mg/m^3. If using a fixed wing aerial application of chlorpyrifos with 2 gallons/acre finished spray volume and 2 lbs/acre application rate as its standard exposure scenario (the most common aircraft used for aerial applications in California and a reasonable "worst case" scenario), and comparing to inhalation RfCs for children 1-2 years old based on the DNT endpoint, this assessment has concluded that modeled air concentrations at all distances exceed the RfC/10 TAC designated air concentration of 0.0005 mg/m^3. See Table 29 below.

Table 29. Modeled Spray Drift Air Concentrations (1hr TWA) of Chlorpyrifos Compared with the Reference Concentration/10 for a Child 1-2 Years Old based on a the Developmental Neurotoxicity Endpoint

Downwind Distance (ft)	1-hr TWA Modeled Air Concentrations (mg/m3)	RfC/10 for a Child 1-2 years old (mg/m3) [TAC designation]
25	0.0493	>0.0005
50	0.0437	
100	0.035	
250	0.0237	
500	0.0153	
1000	0.0072	
1320	0.00492	
2608	0.00163	

CONCLUSION

HHA's comprehensive human health risk assessment involved rigorous analysis of results from in vivo and in vitro experiments, computational toxicology, epidemiology, diet and drinking water assessments, pesticide illness reports, and exposure analysis and modeling in order to determine the risks from exposure to chlorpyrifos. In the December 2017 Draft TAC Evaluation (Appendix 6), HHA reviewed the comprehensive database for AChE inhibition and based the critical PoDs on that parameter. This final TAC evaluation presents a comprehensive analysis of all currently available data to establish a PoD based directly on developmental neurotoxicity.

Available animal studies support the establishment of a PoD based directly on developmental neurotoxicity effects. HHA conducted a comprehensive review of recently available animal studies and focused on the evidence of neurodevelopmental toxicity at low dose levels. Critical PoDs were established from animal studies reporting effects at dose levels that were approximately 10-fold lower than those that inhibit red blood cell AChE. A target MOE of 100 was selected to be protective of human health for the neurodevelopmental endpoint and is comprised of 10x for interspecies sensitivity and 10x for intraspecies variability. There is no need for an additional UF for neurodevelopmental effects. The risk of exposures to inhalation and spray drift is exacerbated by consumption of food and drinking water in this approach. The database for developmental neurotoxicity is evolving, and as new data become available HHA can further refine this assessment.

Adding an additional 10x UF to an AChE inhibition endpoint would indirectly account for the possibility of neurodevelopmental effects, thus increasing the protection factor of the estimated RfC and RfDs for chlorpyrifos. By adding an additional 3x uncertainty factor for PBPK-PD model insufficiencies, the protectiveness in the proposed target RfCs and RfDs has been further increased. The database which supports the AChE endpoint is robust, covering many hundreds of research papers over several decades, with consistency across laboratories and studies for the level of chlorpyrifos that inhibits AChE in red blood cells in both animals and humans. The magnitude of the 10x UF to account for possible developmental effects is well supported by existing data. The use of the AChE inhibition endpoint with the addition of the 10x UF can be considered a surrogate for the more sensitive DNT endpoint.

In conclusion, DPR evaluated the strengths and uncertainties associated with the use of the available database for deriving critical endpoints for chlorpyrifos. Following the recommendation of the SRP, DPR thoroughly evaluated developmental neurotoxicity as the critical endpoint for the chlorpyrifos risk assessment. Based on the evaluation of the toxicity database and exposure analyses, this assessment supports the finding that chlorpyrifos meets the criteria to be listed as a TAC pursuant to the law of California.

REFERENCES

Akassoglou, K., Malester, B., Xu, J., Tessarollo, R., J., and Chao, M. V. 2004. Brain-specific deletion of neuropathy target esterase/swisscheese results in neurodegeneration. Neuroscience 101:5075-5080.

Aldridge, J. E., Meyer, A., Seidler, F. J., and Slotkin, T. A. 2005. Alterations in central nervous system serotonergic and dopaminergic synaptic activity in adulthood after prenatal or neonatal chlorpyrifos exposure. Environ Health Perspect 113:1027-1031.

An, X., Ji, X., Wu, M., Hu, X., Yu, R., Zhao, X., and Cai, L. 2014. Risk assessment of applicators to chlorpyrifos through dermal contact and inhalation at different maize plant heights in China. Journal of Agricultural and Food Chemistry 62:7072-7077.

Bagchi, D., Bagchi, M., Hassoun, E. A., and Stohs, S. J. 1995. In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology 104:129-140.

Barna-Loyd, T., Szabo, J. R., and Young, J. G. 1986. Chlorpyrifos: Subchronic Organophosphate-Induced Delayed-Neurotoxicity (OPIDN) Study In Laying Chicken Hens. Dow Chemical, Freeport, Texas, Study # TXT:K-044793-064 DPR Vol. 342-291 #51119

Barr, D. B., Ananth, C. V., Yan, X., Lashley, S., Smulian, J. C., Ledoux, T. A., Hore, P., and Robson, M. G. 2010. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. The Science of the Total Environment 408:790-795.

Barr, D. B., and Angerer, J. 2006. Potential uses of biomonitoring data: a case study using the organophosphorus pesticides chlorpyrifos and malathion. Environ Health Perspect 114:1763-1769.

Barr, D. B., Barr, J. R., Driskell, W. J., Hill, R. H., Jr., Ashley, D. L., Needham, L. L., Head, S. L., and Sampson, E. J. 1999. Strategies for biological monitoring of exposure for contemporary-use pesticides. Toxicology and Industrial Health 15:168-179.

Barr, D. B., Barr, J. R., Maggio, V. L., Whitehead, R. D., Jr., Sadowski, M. A., Whyatt, R. M., and Needham, L. L. 2002. A multi-analyte method for the quantification of contemporary pesticides in human serum and plasma using high-resolution mass spectrometry. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences 778:99-111.

Barr, J. L., Forster, G. L., and Unterwald, E. M. 2014. Repeated cocaine enhances ventral hippocampal-stimulated dopamine efflux in the nucleus accumbens and alters ventral hippocampal NMDA receptor subunit expression. Journal of Neurochemistry 130:583-590.

Barry, T. A. 2015. Estimation of Chlorpyrifos Horizontal Deposition and Air Concentrations for California Use Scenarios. Memorandum to Kwok, Eric S. C., Human Health Assessment Branch, from Barry, Terri A., Research Scientist IV, dated December 16, 2015. Department of Pesticide Regulation. California Environmental Protection Agency. Sacramento, CA 95812.

Barry, T. A. 2017. Revised: Estimation of Chlorpyrifos Horizontal Deposition and Air Concentrations for California Use Scenarios. Memorandum to Kwok, Eric S. C., Human Health Assessment Branch, from Barry, Terri A., Research Scientist IV, dated August 15, 2017. Department of Pesticide Regulation. California Environmental Protection Agency. Sacramento, CA 95812.

Benowitz, L. I., and Routtenberg, A. 1997. GAP-43: an intrinsic determinant of neuronal development and plasticity. Trends in Neurosciences 20:84-91.

Bielawski, D., Ostrea, E., Jr., Posecion, N., Jr., Corrion, M., and Seagraves, J. 2005. Detection of Several Classes of Pesticides and Metabolites in Meconium by Gas Chromatography-Mass Spectrometry. Chromatographia 62:623-629.

Boix, J., Cauli, O., and Felipo, V. 2010. Developmental exposure to polychlorinated biphenyls 52, 138 or 180 affects differentially learning or motor coordination in adult rats. Mechanisms involved. Neuroscience 167:994-1003.

Bouchard, M. F., Bellinger, D. C., Wright, R. O., and Weisskopf, M. G. 2010. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics 125:1270-1277.

Bradman, A., Whitaker, D., Quiros, L., Castorina, R., Claus Henn, B., Nishioka, M., Morgan, J., Barr, D. B., Harnly, M., Brisbin, J. A., Sheldon, L. S., McKone, T. E., and Eskenazi, B. 2007. Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. Journal of Exposure Science & Environmental Epidemiology 17:331-349.

Brown, T. R., Rumsky, P. C., Capleton, A. C., Rushton, L., and Levy, L. S. 2006. Pesticides and Parkinson's disease - Is there a link? Environ. Health Perspect. 114:156-164.

Buntyn, R. W., Alugubelly, N., Hybart, R. L., Mohammed, A. N., Nail, C. A., Parker, G. C., Ross, M. K., and Carr, R. L. 2017. Inhibition of Endocannabinoid-Metabolizing Enzymes in Peripheral Tissues Following Developmental Chlorpyrifos Exposure in Rats. International Journal of Toxicology 36:395-402.

Burke, R. D., Todd, S. W., Lumsden, E., Mullins, R. J., Mamczarz, J., Fawcett, W. P., Gullapalli, R. P., Randall, W. R., Pereira, E. F. R., and Albuquerque, E. X. 2017. Developmental neurotoxicity of the organophosphorus insecticide chlorpyrifos: from clinical findings to preclinical models and potential mechanisms. J Neurochem 142 Suppl 2:162-177.

Burns, C. J., Cartmill, J. B., Powers, B. S., and Lee, M. K. 1998. Update of the morbidity experience of employees potentially exposed to chlorpyrifos. Occupational and Environmental Medicine 55:65-70.

Burns, C. J., McIntosh , L. J., Mink , P. J., Jurek, A. M., and Li, A. A. 2013. Pesticide Exposure and Neurodevelopmental Outcomes: Review of the Epidemiologic and Animal Studies. Journal of Toxicology and Environmental Health, Part B 16:127-283.

Byrne, S. L., Shurdut, B. A., and Saunders, D. G. 1998. Potential chlorpyrifos exposure to residents following standard crack and crevice treatment. Environmental Health Perspectives 106:725.

Callahan, C. L., Al-Batanony, M., Ismail, A. A., Abdel-Rasoul, G., Hendy, O., Olson, J. R., Rohlman, D. S., and Bonner, M. R. 2014. Chlorpyrifos exposure and respiratory health among adolescent agricultural workers. International Journal of Environmental Research and Public Health 11:13117-13129.

Callahan, C. L., Hamad, L. A., Olson, J. R., Ismail, A. A., Abdel-Rasoul, G., Hendy, O., Rohlman, D. S., and Bonner, M. R. 2017. Longitudinal assessment of occupational determinants of chlorpyrifos exposure in adolescent pesticide workers in Egypt. International Journal of Hygiene and Environmental Health 220:1356-1362.

Capodicasa, E., Scpellato, M. L., Moretto, A., Caroldi, S., and Lotti, M. 1991. Chlorpyrifos-induced delayed polyneuropathy. Arch. Toxicol. 65:150-155.

Carr, R. L., Armstrong, N. H., Buchanan, A. T., Eells, J. B., Mohammed, A. N., Ross, M. K., and Nail, C. A. 2017. Decreased anxiety in juvenile rats following exposure to low levels of chlorpyrifos during development. Neurotoxicology 59:183-190.

Casida, J. E., and Quistad, G. B. 2005. Serine hydrolase targets of organophosphorus toxicants. Chem. Biol. Interact. 157-158:277-283.

Caughlan, A., Newhouse, K., Namgung, U., and Xia, Z. 2004. Chlorpyrifos induces apoptosis in rat cortical neurons that is regulated by a balance between p38 and ERK/JNK MAP kinases. Toxicol. Sci. 78:125-134.

Chiu, C.-S., Brickley, S., Jensen, K., Southwell, A., Mckinney, S., Cull-Candy, S., Mody, I., and Lester, H. A. 2005. GABA transporter deficiency causes tremor, ataxia, nervousness, and increased GABA-induced tonic conductance in cerebellum. Journal of Neuroscience 25:3234-3245.

Chuang, C. S., Su, H. L., Lin, C. L., and Kao, C. H. 2017. Risk of Parkinson disease after organophosphate or carbamate poisoning. Acta Neurol. Scand. 136:129-137.

Corrion, M. L., Ostrea, E. M., Jr., Bielawski, D. M., Posecion, N. C., Jr., and Seagraves, J. J. 2005. Detection of prenatal exposure to several classes of environmental toxicants and

their metabolites by gas chromatography-mass spectrometry in maternal and umbilical cord blood. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 822:221-229.

Coulston, F., Golberg, L., Abraham, K. B., F, Griffin, B., and Norvell, M. 1971. Final Report on Safety Evaluation and Metabolic Studies on DOWCO 179 (IN 151). DPR Volume 342-284.

Dawson, L. J., Britton, W., Bohaty, R., Mallampalli, N., and Grube, A. 2012. Chlorpyrifos: Evaluation of the Potential Risks from Spray Drift and the Impact of Potential Risk Reduction Measures. Memorandum to Wolf, Joel Pesticide Re-Evaluation Division (7508P), from Dawson, L. Jeffrey, Bohaty, Rochelle, Mallampalli, Nikhil, dated July 13. http://www.regulations.gov/#!docketDetail;D=EPA-HQ-OPP-2008-0850.

Debost-Legrand, A., Warembourg, C., Massart, C., Chevrier, C., Bonvallot, N., Monfort, C., Rouget, F., Bonnet, F., and Cordier, S. 2016. Prenatal exposure to persistent organic pollutants and organophosphate pesticides, and markers of glucose metabolism at birth. Environmental research 146:207-217.

Detweiler, M. B. 2014. Organophosphate intermediate syndrome with neurological complications of extrapyramidal symptoms in clinical practice. J. Neurosci. Rural Pract. 5:298-301.

Devici, H. A., and Karapehlivan, M. 2018. Chlorpyrifos-induced parkinsonian model in mice: Behavior, histopathology and biochemistry. Pest. Biochem. Physiol. 144:36-41.

Dhillon, A. S., Tabutton, G. L., Levin, J. L., Plotkin, G. M., Lowry, L. K., Nalbone, J. T., and Shepard, S. 2008. Pesticide/environmental exposures and Parkinson's disease in East Texas. J. Agromed. 13:37-48.

Dodd, C. A., and Klein, B. G. 2009. Pyrethroid and organophosphate insecticide exposure in the 1-methyl-4-pheny-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease: an immunohistochemical analysis of tyrosine hydroxylase and glial fibrillary acidic protein in dorsolateral striatum. Toxicol. Ind. Health 25:25-39.

DPR. 2000. Interim Guidance for Selecting Default Inhalation Rates for Children and Adults. (December 1). 8. Memorandum. http://www.cdpr.ca.gov/docs/whs/memo/hsm00010.pdf.

Eaton, D. L., Daroff, R. B., Autrup, H., Bridges, J., Buffler, P., Costa, L. G., Coyle, J., McKhann, G., Mobley, W. C., Nadel, L., Neubert, D., Schulte-Hermann, R., and Spencer, P. S. 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Critical Reviews in Toxicology 38 Suppl 2:1-125.

Eddleston, M., Singh, S., and Buckley, N. 2007. Organophosphorus poisoning (acute). BMJ Clinical Evidence 2007.

Eells, J. B., and Brown, T. 2009. Repeated developmental exposure to chlorpyrifos and methyl parathion causes persistent alterations in nicotinic acetylcholine subunit mRNA expression with chlorpyrifos altering dopamine metabolite levels. Neurotoxicol. Teratol. 31:98-103.

EFSA 2017. Investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia. EFSA Journal 15:1-325.

Ehrlich, I., and Malinow, R. 2004. Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. Journal of Neuroscience 24:916-927.

Fang, B., Li, J. W., Zhang, M., Ren, F. Z., and Pang, G. F. 2018. Chronic chlorpyrifos exposure elicits diet-specific effects on metabolism and the gut microbiome in rats. Food and Chemical Toxicology 111:144-152.

Farahat, F. M., Ellison, C. A., Bonner, M. R., McGarrigle, B. P., Crane, A. L., Fenske, R. A., Lasarev, M. R., Rohlman, D. S., Anger, W. K., Lein, P. J., and Olson, J. R. 2011. Biomarkers of Chlorpyrifos Exposure and Effect in Egyptian Cotton Field Workers. Environ Health Perspect 119:801-806.

Fenske, R. A., Lu, C., Negrete, M., and Galvin, K. 2013. Breaking the take home pesticide exposure pathway for agricultural families: workplace predictors of residential contamination. Am J Ind Med 56.

Fieten, K. B., Kromhout, H., Heederik, D., and Van Wendel de Joode, B. 2009. Pesticide exposure and respiratory health of indigenous women in Costa Rica. American journal of epidemiology 169:1500-1506.

Fluegge, K. R., Nishioka, M., and Wilkins, J. R., 3rd 2016. Effects of simultaneous prenatal exposures to organophosphate and synthetic pyrethroid insecticides on infant neurodevelopment at three months of age. Journal of environmental toxiology and public health 1:60-73.

Fortenberry, G. Z., Meeker, J. D., Sanchez, B. N., Barr, D. B., Panuwet, P., Bellinger, D., Schnaas, L., Solano-Gonzalez, M., Ettinger, A. S., Hernandez-Avila, M., Hu, H., and Tellez-Rojo, M. M. 2014. Urinary 3,5,6-trichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: distribution, temporal variability, and relationship with child attention and hyperactivity. International journal of hygiene and environmental health 217:405-412.

Freire, C., and Koifman, S. 2012. Pesticide exposure and Parkinson's diease: Epidemiological evidence of association. NeuroToxicology 33:947-971.

French, L. R., Schuman, L. M., Mortimer, J. A., Hutton, J. T., Boatman, R. A., and Christians, B. 1985. A case-control study of dementia of the Alzheimer's type. Am. J. Epidemiol. 121:414-421.

Gao, B., Tao, C., Ye, J., Ning, J., Mei, X., Jiang, Z., Chen, S., and She, D. 2014. Measurement of operator exposure to chlorpyrifos. Pest management science 70:636-641.

Garcia, S. J., Seidler, F. J., and Slotkin, T. A. 2005. Developmental neurotoxicity of chlorpyrifos: targeting glial cells. Environ. Toxicol. Pharmacol. 19:455-461.

Gatto, N. M., Cockburn, M., Bronstein, J., Manthripragada, A. D., and Ritz, B. 2009. Well-water consumption and Parkinson's disease in rural California. Environ. Health Perspect. 117:1912-1918.

Gendron, T. F., and Petrucelli, L. 2009. The role of tau in neurodegeneration. Mole. Neurodegen. 4:1-19.

Gibbs, J. L., Yost, M. G., Negrete, M., and Fenske, R. A. 2017. Passive Sampling for Indoor and Outdoor Exposures to Chlorpyrifos, Azinphos-Methyl, and Oxygen Analogs in a Rural Agricultural Community. Environ Health Perspect 125:333-341.

Goel, D., Singhal, A., Srivastav, R. K., Verma, A., and Lamba, A. 2006. Magnetic resonance imaging changes in a case of extra-pyramidal syndrome after acute organophosphate poisoning. Neurol. India 54:207-208.

Gómez-Giménez, B., Felipo, V., Cabrera-Pastor, A., Agustí, A., Hernández-Rabaza, V., and Llansola, M. 2018. Developmental Exposure to Pesticides Alters Motor Activity and Coordination in Rats: Sex Differences and Underlying Mechanisms. Neurotoxicity research1-12.

Gómez-Giménez, B., Llansola, M., Hernandez-Rabaza, V., Cabrera-Pastor, A., Malaguarnera, M., Agusti, A., and Felipo, V. 2017. Sex-dependent effects of developmental exposure to different pesticides on spatial learning. The role of induced neuroinflammation in the hippocampus. Food and Chemical Toxicology 99:135-148.

Grigoryan, H., and Lockridge, O. 2009. Nanoimages show disruption of tubulin polymerization by chlorpyrifos oxon; implication for neurotoxicity. Toxicol. Appl. Pharmacol. 240:143-148.

Grigoryan, H., Schopfer, L. M., Peeples, E. S., Duysen, E. G., Grigoryan, M., Thompson, C. M., and Lockridge, O. 2009. Mass spectrometry identifies multiple organophosphorylated sites on tubulin. Toxicol. Appl. Pharmacol. 240:149-158.

Gunier, R. B., Nuckols, J. R., Whitehead, T. P., Colt, J. S., Deziel, N. C., Metayer, C., Reynolds, P., and Ward, M. H. 2016. Temporal Trends of Insecticide Concentrations in Carpet Dust in California from 2001 to 2006. Environ Sci Technol 50:7761-7769.

Hanchar, H. J., Dodson, P. D., Olsen, R. W., Otis, T. S., and Wallner, M. 2005. Alcohol-induced motor impairment caused by increased extrasynaptic GABA(A) receptor activity. Nat Neurosci 8:339-345.

Harnly, M. E., Bradman, A., Nishioka, M., McKone, T. E., Smith, D., McLaughlin, R., Kavanagh-Bair, G., Castorina, R., and B., E. 2009. Pesticides in dust from homes in an agricultural area. Environ Sci Technol 43:8767-8774.

Hayden, K. M., Norton, M. C., Darcey, D., Ostbye, T., Zandi, P. P., Breitner, J. C. S., and Welsh-Bohmer, K. A. 2001. Occupational exposure to pesticides increase the risk of incident AD. Neurology 74:1524-1530.

Hoberman, A. M. 1998. Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®(SD)BR VAF/Plus® presumed pregnant rats. *Argus Research Laboratories, Inc., Study # 304-001, Protocol # K-044793-109;* DPR Vol. 342-746 #162521.

Holland, N., Lizarraga, D., and Huen, K. 2015. Recent progress in the genetics and epigenetics of paraoxonase: why it is relevant to children's environmental health. Current opinion in pediatrics 27:240-247.

Hoppin, J. A., Umbach, D. M., London, S. J., Alavanja, M. C., and Sandler, D. P. 2002. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. American Journal of Respiratory and Critical care medicine 165:683-689.

Hoppin, J. A., Umbach, D. M., London, S. J., Lynch, C. F., Alavanja, M. C., and Sandler, D. P. 2006a. Pesticides and adult respiratory outcomes in the agricultural health study. Annals of the New York Academy of Sciences 1076:343-354.

Hoppin, J. A., Umbach, D. M., London, S. J., Lynch, C. F., Alavanja, M. C., and Sandler, D. P. 2006b. Pesticides associated with wheeze among commercial pesticide applicators in the Agricultural Health Study. American journal of epidemiology 163:1129-1137.

Hoppin, J. A., Umbach, D. M., Long, S., London, S. J., Henneberger, P. K., Blair, A., Alavanja, M., Freeman, L. E. B., and Sandler, D. P. 2017. Pesticides Are Associated with Allergic and Non-Allergic wheeze among Male Farmers. Environmental health perspectives 125:535.

Hsieh, B., Deng, J., Ger, J., and Tsai, W. 2001. Acetylcholinesterase inhibition and the extrapyramidal syndrome: a review of the neurotoxicity of organophosphate. Neurotoxicology 22:423-427.

Huen, K., Bradman, A., Harley, K., Yousefi, P., Barr, D. B., Eskenazi, B., and Holland, N. 2012. Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. Environmental Research 117:8-16.

Huen, K., Harley, K., Beckman, K., Eskenazi, B., and Holland, N. 2013. Associations of PON1 and genetic ancestry with obesity in early childhood. PloS one 8:e62565.

Iqbal, K., Grunkle-Iqbal, I., Zaidi, T., Merz, P. A., Wen, G. Y., Shaikh, S. S., Wisniewski, H. M., Alafuzoff, I., and Winblad, B. 1986. Defective brain microtubule assembly in Alzheimer's disease. Lancet 2:421-426.

Ismail, A. A., Bonner, M. R., Hendy, O., Abdel Rasoul, G., Wang, K., Olson, J. R., and Rohlman, D. S. 2017a. Comparison of neurological health outcomes between two adolescent cohorts exposed to pesticides in Egypt. PLoS One 12:e0172696.

Ismail, A. A., Wang, K., Olson, J. R., Bonner, M. R., Hendy, O., Abdel Rasoul, G., and Rohlman, D. S. 2017b. The impact of repeated organophosphorus pesticide exposure on biomarkers and neurobehavioral outcomes among adolescent pesticide applicators. Journal of toxicology and environmental health. Part A 80:542-555.

Jakel, S., and Dimou, L. 2017. Glial cells and their function in the adult brain: A journey through the history of their ablation. Front. Cell. Neurosci. 11:Article 24.

Jiang, W., Duysen, E. G., Hansen, H., Shlyakhtenko, L., Schopfer, L. M., and Lockridge, O. 2010. Mice treated with chlorpyrifos or chlorpyrifos oxon have organophosphorylated tubulin in the brain and disrupted micotubule structures, suggesting a role for tubulin in neurotoxicity associated with exposure to organophosphorus agents. Toxicol. Sci. 115:183-193.

Kamel, F., Tanner, C. M., Umbach, D. M., Hoppin, J. A., Alavanja, M. C. R., Blair, A., Comyns, K., Goldman, S. M., Korell, M., Langston, J. W., Ross, G. W., and Sandler, D. P. 2006. Pesticide exposure and self-reported Parkinson's disease in the agricultural health study Am. J. Epidemiol. 165:364-374.

Karalliedde, L., Baker, D., and Marrs, T. C. 2006. Organophosphate-induced intermediate syndrome - aetiology and relationships with myopathy. Toxicol. Rev. 25:1-14.

Karen, D. J., Li, W., Harp, P. R., Gillette, J. S., and Bloomquist, J. R. 2001. Striatal dopaminergic pathways as a target for the insecticides permethrin and chlorpyrifos. NeuroToxicology 22:811-817.

Kou, J., Gillette, J. S., and Bloomquist, J. R. 2006. Neurotoxicity in murine striatal dopaminergic pathways following co-application of permethrin, chlorpyrifos and MPTP. Pest. Biochem. Physiol. 85:68-75.

Kwon, O. D., and Kim, H. K. 2014. Parkinsonism as late sequela of organophosphate intoxication. J. Emerg. Trauma Shock 7:124-125.

LaKind, J. S., Sobus, J. R., Goodman, M., Barr, D. B., Furst, P., Albertini, R. J., Arbuckle, T. E., Schoeters, G., Tan, Y. M., Teeguarden, J., Tornero-Velez, R., and Weisel, C. P. 2014. A

proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. Environ Int 73:195-207.

Lassiter, T. L., and Brimijoin, S. 2008. Rats gain excess weight after developmental exposure to the organophosphorothionate pesticide, chlorpyrifos. Neurotoxicol Teratol 30:125-130.

Lee, I., Eriksson, P., Fredriksson, A., Buratovic, S., and Viberg, H. 2015. Developmental neurotoxic effects of two pesticides: Behavior and biomolecular studies on chlorpyrifos and carbaryl. Toxicology and Applied Pharmacology 288:429–438.

Lee, P.-C., Rhodes, S. L., Sinsheimer, J. S., Bronstein, J., and Ritz, B. 2013. Functional paraoxonase 1 variants modify the risk of Parkinson's disease due to organophosphate exposure. Environ. Int. 56:42-47.

Lee, S., McLaughlin, R., Harnly, M., Gunier, R., and Kreutzer, R. 2002. Community exposures to airborne agricultural pesticides in California: ranking of inhalation risks. Environmental Health Perspectives 110:1175.

Lee, Y. S., Lewis, J. A., Ippolito, D. L., Hussainzada, N., Lein, P. J., Jackson, D. A., and Stallings, J. D. 2016. Repeated exposure to neurotoxic levels of chlorpyrifos alters hippocampal expression of neurotrophins and neuropeptides. Toxicology 340:53-62.

Lewis, R. G., Fortune, C. R., Blanchard, F. T., and DE., C. 2001. Movement and Deposition of Two Organophosphorus Pesticides within a Residence after Interior and Exterior Applications. Journal of the Air & Waste Management Association 51:339-351.

Lotti, M., Bentoncin, D., and Moretto, A. 1986a. Organophosphate induced delayed polyneuropathy (OPIDP) by chlorpyrifos in man and hens. Toxicologist 6:22.

Lotti, M., Moretto, A., Zoppellari, R., Dainese, R., Rizzuto, N., and Barusco, G. 1986b. Inhibition of lymphocytic neuropathy target esterasee predicts the development of organophosphate-induced delayed polyneuropathy. Arch. Toxicol. 59:176-179.

Manthripragada, A. D., Costello, S., Cockburn, M., Bronstein, J., and Ritz, B. 2010a. Paraoxonase 1, agricultural organophosphate exposure and Parkinson disease. Epidemilogy 21:87-94.

Manthripragada, A. D., Costello, S., Cockburn, M. G., Bronstein, J. M., and Ritz, B. 2010b. Paraoxonase 1 (PON1), agricultural organophosphate exposure, and Parkinson disease. Epidemiology (Cambridge, Mass.) 21:87.

Masoud, A., Kiran, R., and Sandhir, R. 2009. Impaired mitochondrial functions in organophosphate induced delayed neuropathy in rats. Cell. Mol. Neurobiol. 29:1245-1255.

Masscotte, C., Knight, K., van der Schyf, C., Jortner, B. S., and Ehrich, M. 2005. Effects of organophosphate compounds on ATP production and mitochondiral integrity in cultured cells. Neurotox. Res. 7:203-217.

Meggs, W.J. and Brewer, K.L. (2007). Weight gain associated with chronic exposure to chlorpyrifos in rats. Journal of Medical Toxicology, 3 (3), 89-93.Mendes, P. A., Pereira, T. C., Pina, R., and Santos, R. 2017. Chlorpyrifosinduces delayed neurotoxicity with a rare presentation of flaccid quadriplegia: a diagnostic challenge. Eur. J. Case Rep. Intern. Med. 4.

Mendes, P. A., Pereira, T. C., Pina, R., and Santos, R. 2017. Chlorpyrifosinduces delayed neurotoxicity with a rare presentation of flaccid quadriplegia: a diagnostic challenge. Eur. J. Case Rep. Intern. Med. 4.

Middlemore-Risher, M.-L., Adam, B.-L., Lambert, N. A., and Terry, A. V. 2011. Effects of chlorpyrifos and chlorpyrifos-oxon on the dynamics and movement of mitochondria in rat cortical neurons J. Pharmacol. Exp. Therap. 339:341-349.

Mohammed, A. N., Armstrong, N. H., Buchanan, A. T., Eells, J. B., Ross, M. K., Nail, C. A., and Carr, R. L. 2015. Altered Emotional Reactivity and Dopamine Turnover in Juvenile Rats Exposed Developmentally to Chlorpyrifos. The Toxicologist (Supplement to Toxicological Sciences) available at www.toxicology.org 144:457.

Moreno, M., Cañadas, F., Cardona, D., Suñol, C., Campa, L., Sánchez-Amate, C., Flores, P., and Sanchez-Santed, F. 2008. Long-term monoamine changes in the striatum and nucleus accumbens after acute chlorpyrifos exposure. Toxicol. Lett. 176:162-167.

Morfini, G. A., Burns, M., Binder, L., Kanaan, N. M., LaPointe, N., Bosco, D. A., Brown Jr, R. H., Brown, H., Tiwari, A., Hayward, L., Edgar, J., Nave, K.-A., Garberrn, J., Atagi, Y., Song, Y., Pigino, G., and Brady, S. T. 2009. Axonal transport defects in neurodegenerative diseases. J. Neurosci. 29:12776-12786.

Muller, M., Hess, L., Tardivo, A., Lajmanovich, R., Attademo, A., Poletta, G., Simoniello, M. F., Yodice, A., Lavarello, S., and Chialvo, D. 2014. Neurologic dysfunction and genotoxicity induced by low levels of chlorpyrifos. Neurotoxicology 45:22-30.

Munoz-Quezada, M. T., Lucero, B., Iglesias, V., Levy, K., Munoz, M. P., Achu, E., Cornejo, C., Concha, C., Brito, A. M., and Villalobos, M. 2017. Exposure to organophosphate (OP) pesticides and health conditions in agricultural and non-agricultural workers from Maule, Chile. International Journal of Environmental Health Research 27:82-93.

Nand, N., Aggarwal, H. K., Bharti, K., and Chakarabarti, D. 2007. Organophosphate induced delayed neuropathy. J. Assoc. Phys. India 55:72-73.

Narayan, S., Liew, Z., Paul, K., Lee, P.-C., Sinsheimer, J. S., Bronstein, J., and Ritz, B. 2013. Household organophosphate pesticude use and Parkinson's disease. Intern. J. Epidemiol. 42:1476-1485.

Navone, F., Jahn, R., Di Gioia, G., Stukenbrok, H., Greengard, P., and De Camilli, P. 1986. Protein p38: an integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. The Journal of cell biology 103:2511-2527.

Neta, G., Goldman, L. R., Barr, D., Sjodin, A., Apelberg, B. J., Witter, F. R., and Halden, R. U. 2010. Distribution and determinants of pesticide mixtures in cord serum using principal component analysis. Environ Sci Technol 44:5641-5648.

Nielson, S. S., Checkoway, H., Zhang, J., Hofmann, J. N., Keifer, M. C., Paulsen, M., Farin, F. M., Cook, T. J., and Simpson, C. D. 2015. Blood α–synuclein in agricultural pesticide handlers in central Washington state. Environ. Res. 136:78-81.

O'Callaghan, J. P., and Sriram, K. 2005. Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. Expert Opin. Drug Saf. 4:433-443.

OEHHA. 2012. Air Toxics Hot Spots Program Risk Assessment Guidelines: Technical Support Document For Exposure Assessment And Stochastic Analysis. OEHHA In: Air Toxics Hot Spots Program https://oehha.ca.gov/air/air-toxics-hot-spots.

Ostrea, E. M., Jr., Bielawski, D. M., Posecion, N. C., Jr., Corrion, M., Villanueva-Uy, E., Bernardo, R. C., Jin, Y., Janisse, J. J., and Ager, J. W. 2009. Combined analysis of prenatal (maternal hair and blood) and neonatal (infant hair, cord blood and meconium) matrices to detect fetal exposure to environmental pesticides. Environ Res 109:116-122.

Ostrea, E. M., Jr., Reyes, A., Villanueva-Uy, E., Pacifico, R., Benitez, B., Ramos, E., Bernardo, R. C., Bielawski, D. M., Delaney-Black, V., Chiodo, L., Janisse, J. J., and Ager, J. W. 2012. Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. Neurotoxicology 33:669-675.

Ostrea, E. M., Jr., Villanueva-Uy, E., Bielawski, D. M., Posecion, N. C., Jr., Corrion, M. L., Jin, Y., Janisse, J. J., and Ager, J. W. 2006. Maternal hair--an appropriate matrix for detecting maternal exposure to pesticides during pregnancy. Environ Res 101:312-322.

Ostwal, P., Dabadghao, V. S., Sharma, S. K., and Dhakane, A. B. 2013. Chlorpyrifos toxicity causing delayed myeloneuropathy. Ann. Indian Acad. Neurol. 16:736.

Pallotta, M. M., Ronea, R., Carotenuto, R., Porreca, I., Turano, M., Ambrosino, C., and Capriglione, T. 2017. Specific effects of chronic dietary exposure to chlorpyrifos on brain gene expression - a mouse study. Int. J. Mol. Sci. 18.

Panda, A. K., Bala, K., and Bhirud, L. 2014. Extrapyramidal syndrome. BMJ Case Rep.1-4.

Peleg-Raibstein, D., and Feldon, J. 2006. Effects of dorsal and ventral hippocampal NMDA stimulation on nucleus accumbens core and shell dopamine release. Neuropharmacology 51:947-957.

Perera, F. P., Rauh, V., Whyatt, R., Tang, D., Tsai, W., Bernert, J., Tu, Y., Andrews, H., Barr, D., and Camann, D. 2005. A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticide exposures. Neurotoxicology 26:573-587.

Perez, J. J., Williams, M. K., Weerasekera, G., Smith, K., Whyatt, R. M., Needham, L. L., and Barr, D. B. 2010. Measurement of pyrethroid, organophosphorus, and carbamate insecticides in human plasma using isotope dilution gas chromatography-high resolution mass spectrometry. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 878:2554-2562.

Peris-Sampedro, F., et al. (2015). Adulthood dietary exposure to a common pesticide leads to an obese-like phenotype and a diabetic profile in apoE3 mice. Environmental research, 142, 169-76.

Poet, T. S., Timchalk, C., Bartels, M. J., Smith, J. N., McDougal, R., Juberg, D. R., and Price, P. S. 2017. Use of a probabilistic PBPK/PD model to calculate Data Derived Extrapolation Factors for chlorpyrifos. Regulatory Toxicology and Pharmacology 86:59-73.

Posecion, N., Jr., Ostrea, E., Jr., Bielawski, D., Corrion, M., Seagraves, J., and Jin, Y. 2006. Detection of Exposure to Environmental Pesticides During Pregnancy by the Analysis of Maternal Hair Using GC-MS. Chromatographia 64:681-687.

Prendergast, M. A., Self, R. L., Smith, K. J., Ghayoumi, L., Mullins, M. M., Butler, T. R., Buccafusco, J. J., Gearhart, D. A., and Terry, A. V., Jr. 2007. Microtubule-associated targets in chlorpyrifos oxon hippocampal neurotoxicity. Neuroscience 146:330-339.

Putnam, R. A., Doherty, J. J., and Clark, J. M. 2008. Golfer exposure to chlorpyrifos and carbaryl following application to turfgrass. Journal of agricultural and food chemistry 56:6616-6622.

Qiao, D., Seidler, F. J., and Slotkin, T. A. 2005. Oxidative mechanism contributing the developmental neurotoxicity of nicotine and chlorpyrifos. Toxicol. Appl. Pharmacol. 206:17-26.

Quiros-Alcala, L., Bradman, A., M., N., Harnly, M. E., A., H., McKone, T. E., Ferber, J., and B., E. 2011. Pesticides in house dust from urban and farmworker households in California: an observational measurement study. Environ Health 10:19.

Raanan, R., Harley, K. G., Balmes, J. R., Bradman, A., Lipsett, M., and Eskenazi, B. 2015. Early-life Exposure to Organophosphate Pesticides and Pediatric Respiratory Symptoms in the CHAMACOS Cohort. Environmental Health Perspectives 123:179-185.

Rainer, S., Bui, M., Mark, E., Thomas, D., Tokarz, D., Ming, L., Delaney, C., Richardson, J. R., Albers, J. W., Matsunami, N., Stevens, J., Coon, H., Leppert, M., and Fink, J. K. 2008. Neuropahty target esterase gene mutations cause motor neuron disease. Am. J. Hum. Genet. 82:780-785.

Ranjbar, M., Rotondi, M. A., Ardern, C. I., and Kuk, J. L. 2015. The influence of urinary concentrations of organophosphate metabolites on the relationship between BMI and cardiometabolic health risk. Journal of obesity 2015.

Read, D. J., Li, Y., Chao, M. V., Cavanagh, J. B., and Glynn, P. 2009. Neuropathy target esterase is required for adult vertebrate axon maintenance. J. Neurosci. 29:11594-11600.

Reygner, J., Joly Condette, C., Bruneau, A., Delanaud, S., Rhazi, L., Depeint, F., Abdennebi-Najar, L., Bach, V., Mayeur, C., and Khorsi-Cauet, H. 2016. Changes in Composition and Function of Human Intestinal Microbiota Exposed to Chlorpyrifos in Oil as Assessed by the SHIME((R)) Model. International journal of environmental research and public health 13.

Richardson, J. R., Hein, N. D., Wijeyesakere, S. J., Fink, J. K., and Makhaeva, G. F. 2013. Neuropathy target esterase (NTE): overview and future. Chem. Biol. Interact. 203:238-244.

Richardson, R. J., Moore, T. B., Kayyali, U. S., Fowke, J. H., and Randall, J. C. 1993a. Inhibition of hen brain acetylcholinesterase and neurotoxici esterase by chlorpyrifos in vivo and kinetics by chlorpyrifos oxon in vitro: Application to assessment of neuropathic risk. Fund. Appl. Toxicol. 20:273-279.

Richardson, R. J., Moore, T. B., Kayyali, U. S., and Randall, J. C. 1993b. Chlorpyrifos: Assessment of potential for delayed neurotoxicity by repeated dosing in adult hens with monitoring of brain acetylcholinesterase, braina and lymphocyte neurotoxic esterase and plasma butyrylcholinesterase activities. Fund. Appl. Toxicol. 21:89-96.

Ritz, B. R., Paul, K. C., and Bronstein, J. M. 2016. Of pesticides and men: A California story of genes and environment in Parkinson's disease. Curr. Environ. Heath Rpt. 3:40-52.

Rohlman, D. S., Ismail, A. A., Rasoul, G. A., Bonner, M. R., Hendy, O., Mara, K., Wang, K., and Olson, J. R. 2016. A 10-month prospective study of organophosphorus pesticide exposure and neurobehavioral performance among adolescents in Egypt. Cortex; a journal devoted to the study of the nervous system and behavior 74:383-395.

Rongo, C., and Kaplan, J. M. 1999. CaMKII regulates the density of central glutamatergic synapses in vivo. Nature 402:195.

Ross, S. M., McManus, I. C., Harrison, V., and Mason, O. 2013. Neurobehavioral problems following low-level exposure to organophosphate pesticides: a systematic and meta-analytic review. Critical Reviews in Toxicology 43:21-44.

Ruiz-Muñoz, A., Nieto-Escamez, F. A., Aznar, S., Colomina, M. T., and Sanchez-Santed, F. 2011. Cognitive and histological disturbances after chlorpyrifos exposure and chronic Aβ(1-42) infusions in Wistar rats. NeuroToxicol. 32:836-844.

Salama, M., El-Morsy, D., El-Gamal, M., Shabka, O., and Mohamed, W. M. Y. 2014. Mitochondrial complex I inhibition as a possible mechanism of chlorpyrifos induced neurotoxicity. Ann. Neurosci. 21:85-89.

Salazar, J. G., Ribes, D., Cabre, M., Domingo, J. L., Sanchez-Santed, F., and Colomina, M. T. 2011. Amyloid β peptide levels increase in brain of AβPP Swedish mice after exposure to chlorpyrifos. Curr. Alzheimer Res. 8:732-740.

Samsam, T. E., Hunter, D. L., and Bushnell, P. J. 2005. Effects of chronic dietary and repeated acute exposure to chlorpyrifos on learning and sustained attention in rats. Toxicol Sci 87:460-468.

Seidler, F. J., and Slotkin, T. A. 2011. Developmental neurotoxicity targeting hepatic and cardiac sympathetic innervation: Effects of organophosphates are distinct from those of glucocorticoids. Brain Research Bulletin 85:225-230.

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., and Noble-Haeusslein, L. J. 2013. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Progress in Neurobiology 106:1-16.

Silva, J. G., Boaretob, A. C., Schreiberb, A. K., Redivob, D. D. B., Gambetab, E., Vergarab, F., Moraisb, H., Zanoveli, J. M., and Dalsenter, P. R. 2017. Chlorpyrifos induces anxiety-like behavior in offspring rats exposed during pregnancy. Neuroscience Letters 641:94-100.

Silver, M. K., Shao, J., Chen, M., Xia, Y., Lozoff, B., and Meeker, J. D. 2015. Distribution and Predictors of Pesticides in the Umbilical Cord Blood of Chinese Newborns. International Journal of Environmental Research and Public Health 13:94.

Silver, M. K., Shao, J., Ji, C., Zhu, B., Xu, L., Li, M., Chen, M., Xia, Y., Kaciroti, N., Lozoff, B., and Meeker, J. D. 2018. Prenatal organophosphate insecticide exposure and infant sensory function. International Journal of Hygiene and Environmental Health 221:469-478.

Silver, M. K., Shao, J., Zhu, B., Chen, M., Xia, Y., Kaciroti, N., Lozoff, B., and Meeker, J. D. 2017. Prenatal naled and chlorpyrifos exposure is associated with deficits in infant motor function in a cohort of Chinese infants. Environ Int 106:248-256.

Slotkin, T. A., Brown, K. K., and Seidler, F. J. 2005. Developmental Exposure of Rats to Chlorpyrifos Elicits Sex-Selective Hyperlipidemia and Hyperinsulinemia in Adulthood. Environ Health Perspect 113:1291-1294.

Smith, M. N., Workman, T., McDonald, K. M., Vredevoogd, M. A., Vigoren, E. M., Griffith, W. C., Thompson, B., Coronado, G. D., Barr, D., and Faustman, E. M. 2017. Seasonal and occupational trends of five organophosphate pesticides in house dust. Journal of Exposure Science & Environmental Epidemiology 27:372-378.

Stafford, L. E., and Robb, C. K. 1999. Determination of Dislodgeable Foliar Residues on Turf Treated with Formulations Containing Chlorpyrifos. 9330 Zionsville Road, Indianapolis, Indiana 46268-1054: Global Environmental Chemistry Laboratory-Indianapolis Lab, Dow AgroSciences LLC. MRID (DPR Vol. No. 342-0979, Record No. 286891) 133.

Steenland, K., Dick, R. B., Howell, R. J., Chrislip, D. W., Hines, C. J., Reid, T. M., Lehman, E., Laber, P., Krieg, E. F., Jr., and Knott, C. 2000. Neurologic function among termiticide applicators exposed to chlorpyrifos. Environ Health Perspect 108:293-300.

Sweeney, P., Park, H., Baumann, M., Dunlop, J., Frydman, J., Kopito, R., McCampbell, A., LeBlanc, G., Venkateswaran, A., Nurmi, A., and Hodgson, R. 2017. Protein misfolding in neurodegenerative diseases: implications and strategies. Transl. Nuerodegen. 6:1-13.

Terry, A. V., Jr., Stone, J. D., Buccafusco, J. J., Sickles, D. W., Sood, A., and Pendergast, M. A. 2003. Repeated exposures to subthreshold doses of chlorpyrifos in rats: Hippocampal damage, impaired axonal transport, and deficits in spatial learning. J. Pharmacol. Exper. Therapeut. 305:375-384.

Teske, M. E., Bird, S. L., Esterly, D. M., Curbishley, T. B., Ray, S. L., and Perry, S. G. 2002a. AgDrift®: A model for estimating near-field spray drift from aerial applications. Environmental Toxicology and Chemistry 21:659-671.

Teske, M. E., Bird, S. L., Esterly, D. M., Ray, S. L., and Perry, S. G. 2002b. A User's Guide for AgDRIFT® 2.0.05: A Tiered Approach for the Assessment of Spray Drift of Pesticides. Regulatory Version. C.D.I. Report No. 01-02. Prepared for David R. Johnson, Project Manager. Spray drift task force c/o Stewart Agricultural Services, Inc. P.O. Box 509, Macon, Missouri 63552. AgDRIFT® 2151.

Thivakaran, T., Gamage, R., Gunarathne, K. S., and Gooneratne, I. K. 2012. Chlorpyrifos-induced delayed myelopathy and pure motor neuropathy. The Neurologist 18:226-228.

Thongsinthusak T, 1991. Determination of Dermal Absorption of Chlorpyrifos in Humans. Memo from T. Thongsinthusak to M. Black. Department of Pesticide Regulation, Worker Health and Safety Branch, HSM-91002, August 2, 1991. Available at http://www.cdpr.ca.gov/docs/whs/memo/hsm91002.pdf.

Ticozzi, N., LeClerc, A. L., Keagle, P., Glass, J. D., Wills, A.-M., van Blitterswijk, M., Bosco, D. A., Rodriguez-Leyva, I., Gellera, C., Ratti, A. T., F., McKenna-Yasek, D. M., Sapp, P. C., Silani, V., Furlong, C. E., Brown Jr, R. H., and Landers, J. E. 2010. Paraoxonase gene mutations in amyotrophic lateral sclerosis. Ann. Neurol. 6891): 102-107.

Torres-Altoro, M. I., Mathur, B. N., Drerup, J. M., Thomas, R., Lovinger, D. M., O'Callaghan, J. P., and Bibb, J. A. 2011. Organophosphates dysregulate dopamine signaling, glutamatergic neurotransmission, and induce neuronal injury markers in striatum. Journal of neurochemistry 119:303-313.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., and Dingledine, R. 2010. Glutamate receptor ion channels: structure, regulation, and function. Pharmacological reviews 62:405-496.

Tyson, T., Steiner, J. A., and Brundin, P. 2016. Sorting out release, uiptake and processing of alpha-synuclein during prion-like spread of pathology. J. Neurochem. 139:275-289.

US EPA 2011. Preliminary Human Health Risk Assessment for Chlorpyrifos. United States Environmental Protection Agency, Washington D.C.

US EPA 2012a. Standard Operating Procedures for Residential Pesticide Exposure Assessment. In https://www.epa.gov/sites/production/files/2015-08/documents/usepa-opp-hed_residential_sops_oct2012.pdf. U.S. Environmental Protection Agency, Washington, DC, Health Effects Division, Office of Pesticide Programs, Office of Chemical Safety and Pollution Prevention.

US EPA 2012b. Registration Review of Chlorpyrifos (PC Code 059101). Appendix F – Chlorpyrifos – Evaluation of the Potential Risks from Spray Drift and the Impact of Potential Risk Reduction Measures. US Environmental Protection Agency, Office of Pesticide Programs, July 13, 2012.Docket No. EPA-HQ-OPP-2016-0167. Available at https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0850-0107

US EPA 2013. Memorandum Dated Januray 31, 2013. Chlorpyrifos; Preliminary Evaluation of the Potential Risks from Volatilization. *United States Environmental Protection Agency, Washington D.C., Office of Chemical Safety and Pollution Prevention.*

US EPA. 2014. Chlorpyrifos Acute and Steady State Dietary (Food Only) Exposure Analysis to Support Registration Review, November 18, 2014. PC Code: 059101. DP Barcode: D424486.

US EPA 2016a. Chlorpyrifos Issue Paper: Evaluation of Biomonitoring Data from Epidemiology Studies Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC. EPA-HQ-OPP-2016-0062-0005:https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2016-0062-0005.

US EPA/SAP 2016. Transcript of: US Environmental Protection Agency (EPA) FIFRA Scientific Advisory Panel (SAP) Meeting on chlorpyrifos: Analysis of biomonitoring data. U.S. Environmental Protection Agency, Washington, DC.; Meeting held April 19-21, 2016, Arlington, VA. EPA-HQ-OPP-2016-0062.

Vaccaro, J., Nolan, R. J., Murphy, P., and 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).

Velmurugan, G., Ramprasath, T., Swaminathan, K., Mithieux, G., Rajendhran, J., Dhivakar, M., Parthasarathy, A., Babu, D. D., Thumburaj, L. J., Freddy, A. J., Dinakaran, V., Puhari, S. S., Rekha, B., Christy, Y. J., Anusha, S., Divya, G., Suganya, K., Meganathan, B., Kalyanaraman, N., Vasudevan, V., Kamaraj, R., Karthik, M., Jeyakumar, B., Abhishek, A., Paul, E., Pushpanathan, M., Rajmohan, R. K., Velayutham, K., Lyon, A. R., and Ramasamy, S. 2017. Gut microbial degradation of organophosphate insecticides-induces glucose intolerance via gluconeogenesis. Genome Biology 18:8.

Victoria, G. S., and Zurzolo, C. 2017. The spread of prion-like proteins by lysosomes and tunneling nanotubes: Implications for neurodegenerative diseases. J. Cell Biol. 216:2633.

Voorhees, J. R. 2017. Cognitive impairment and neuronal damage in Alzheimer's disease are malleable: occupational chlorpyrifos exposure exacerbates phenotypes, while the neuroprotective compound P7C3 ameliorates effects in a transgenic model of Alzheimer's disease. Human Toxicology Thesis, Univeristy of Iowa, Iowa City, IA.

Wang, A., Cockburn, M., Ly, T. T., Bronstein, J., and Ritz, B. 2014. The association between ambient exposure to organophosphates and Parkinson's disease. Occup. Environ. Med. 71:275-281.

Wang, J.-Z., and Liu, F. 2008. Microtubule-associated protein tau in development, degeneration and protection of neurons. Progress in neurobiology 85:148-175.

Wang, Hui-Ping, et al., 2009. Metabolic profiles of serum from rats after subchronic exposure to chlorpyrifos and carbaryl. Chemical research in toxicology, 22 (6), 1026-33.Whyatt, R. M., Barr, D. B., Camann, D. E., Kinney, P. L., Barr, J. R., Andrews, H. F., Hoepner, L. A., Garfinkel, R., Hazi, Y., Reyes, A., Ramirez, J., Cosme, Y., and Perera, F. P. 2003. Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect 111:749-756.

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

Whyatt, R. M., Garfinkel, R., Hoepner, L. A., Andrews, H., Holmes, D., Williams, M. K., Reyes, A., Diaz, D., Perera, F. P., Camann, D. E., and Barr, D. B. 2009. A biomarker validation study of prenatal chlorpyrifos exposure within an inner-city cohort during pregnancy. Environ Health Perspect 117:559-567.

Wickerham, E. L., Lozoff, B., Shao, J., Kaciroti, N., Xia, Y., and Meeker, J. D. 2012. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environ Int 47:80-85.

Wiedenmann, B., and Franke, W. W. 1985. Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. Cell 41:1017-1028.

Wijeyesakere, S. J., and Richardson, J. R. 2010. Neuropathy Target Esterase, edited by R. Krieger. Amsterdam: Elsevier, pp. 1435-1455.

Winrow, C. J., Hemming, M. L., Allen, D. M., Quistad, G. B., Casida, J. E., and Barlow, C. 2003. Loss of neuropathy target esterase in mice links organophosphate exposure to hyperactivity. Nat. Genet. 33:477-485.

Xue, J., Zartarian, V., Tulve, N., Moya, J., Freeman, N., Auyeung, W., and Beamer, P. 2010. A meta-analysis of children's object-to-mouth frequency data for estimating non-dietary ingestion exposure. Journal of Exposure Science & Environmental Epidemiology 20:536-545.

Yalbuzdag, S. A., Ince, B., Karatepe, A. G., Sengul, I., and Kaya, T. 2017. Organophosphate induced delayed neuropathy: a case report. Turk. J. Phys. Med. Rehab. 63:88-91.

Yamada, S., Kubo, Y., Yamzaki, D., Sekino, Y., and Kanda, Y. 2017. Chlorpyrifos inhibits neural induction via Mfn1-mediated mitochondrial dysfunction in human induced pluripotent stem cells. Sci Rep. 7:1-12.

Yan, C., Jiao, L., Zhao, J., Yang, H., and Peng, S. 2012. Repeated exposures to chlorpyrifos lead to spatial memory retrieval impairment and motor activity alteration. Neurotoxicol Teratol 34:442-449.

Yan, D., Zhang, Y., Liu, L., and Yan, H. 2016. Pesticide exposure and risk of Alzheimer's disease: a systematic review and meta-analysis. Sci Rep. 6:1-9.

Zhang, J., Dai, H., Deng, Y., Tian, J., Zhang, C., Hu, Z., Bing, G., and Zhao, L. 2015. Neonatal chlorpyrifos exposure induces loss of dopaminergic neurons in young adult rats. Toxicology 336:17-25.

Zurlinden, T. J., and Reisfeld, B. 2018. A Novel Method for the Development of Environmental Public Health Indicators and Benchmark Dose Estimation Using a Health-Based End Point for Chlorpyrifos. Environ Health Perspect 126(4):047009-1 to 047009-12.