APPENDIX 6.

Draft Evaluation of Chlorpyrifos as a Toxic Air Contaminant:

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

Revised December 11, 2017

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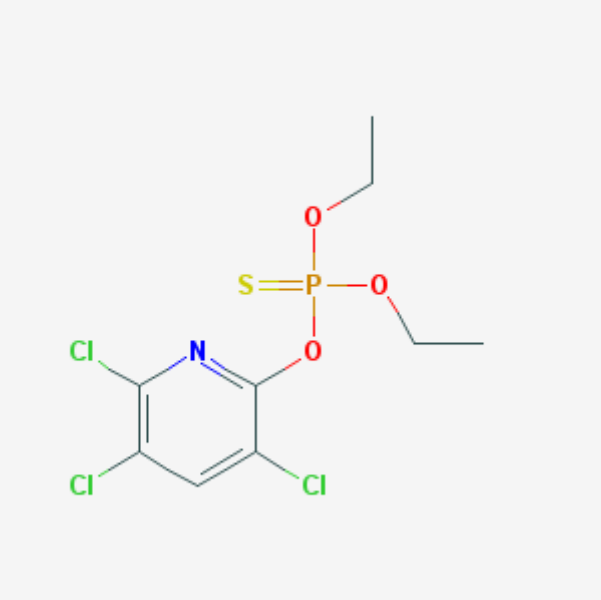

Human Health Assessment Branch
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AADD	Annual average daily dose
AC	Adenylcyclase
AC_{50}	Active concentration resulting in activity of 50% of group
ACh	Acetylcholine
AChE	Acetylcholinesterase
ADD	Absorbed daily dose
AEA	Anandamide
2-AG	2-Arachidonoylglycerol
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
cAMP	Cyclic AMP
CCCEH	Columbia Center for Children's Environmental Health
CES	Carboxyesterase
CNS	Central nervous system
CPF	Chlorpyrifos
CPF-oxon	Chlorpyrifos oxon
DA	Dopamine
DAP	Dialkylphosphate
DPR	California Department of Pesticide Regulation
DOPAC	3,4-Dihydroxyphenylacetic acid
EMON	Environmental Monitoring Branch
FAAH	Fatty acid amide hydrolase
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
5HT	Serotonin
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
MAGL	Monoacylglycerol lipase
MCL	Maximum contaminant level
MDL	Minimal detection limit
MOA	Mode of action
MOE	Margin of exposure
MTD	Maximum tolerated dose
NE	Norepinephrine
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NRDC	National Resources Defense Fund
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
PNS	Peripheral nervous system
RAC	Raw agricultural commodity
RAS	Risk Assessment Section
RBC	Red blood cell
RED	Reregistration eligibility decision
RfD	Reference dose
SADD	Seasonal absorbed daily dose
SAP	Scientific Advisory Panel
s.c.	Subcutaneous
SF	Safety factor
TCPy	3,5,6-trichloro-2-pyridinol
ToxCast	US EPA Toxicity ForeCaster
ToxPi	Toxicological Priority Index
UF	Uncertainty factor
US EPA	US Environmental Protection Agency

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EXECUTIVE SUMMARY

Chlorpyrifos (CPF) is a chlorinated organophosphorus (OP) ester used as an insecticide, acaricide, and miticide. The toxicity of CPF is associated with binding and inhibition of the enzyme acetylcholinesterase (AChE) in insects and mammals. CPF requires metabolic activation to CPF-oxon to yield anticholinesterase activity. CPF may cause developmental neurotoxicity at exposure levels that do not induce overt toxicity or inhibit cholinesterase (ChE) activity.

CPF 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.

This risk assessment addresses potential human effects arising from exposure to CPF from food, drinking water, air and skin contact, incidental ingestion contact, as well as aggregate exposures from various combined scenarios. The health risk assessment was carried out for 4 sentinel subgroups of the general population: infants (<1 year old), children 1-2 years old, children 6-12 years old, and females of childbearing age (13-49 years old). The critical toxicological points of departure (PoDs) used to characterize the risk from exposure to CPF were human equivalent doses estimated by physiologically based pharmacokinetic and pharmacodynamic modeling. Risks were calculated as margin of exposure (MOE), which was equal to the critical PoD divided by the anticipated human exposure level. The Department of Pesticide Regulation (DPR) based its PoDs on the 2014 US EPA Revised Human Health Risk Assessment for CPF. A MOE of 100 was considered protective of human health for all exposure scenarios. The target of 100 included uncertainty factors (UF) of 1 for interspecies sensitivity, 10 for intraspecies variability, and 10 for potential neurodevelopmental effects. Exposures resulting in MOEs lower than the target of 100 are considered to be of potential health risk to humans. DPR's Human Health Assessment Branch (HHA) used the PoDs and the target UF of 100 to estimate reference doses or reference concentrations for chlorpyrifos (Executive Summary Table 1).

Executive Summary Table 1. Points of Departure, Reference Doses, or Concentrations used to Evaluate the Risk from Various Single and Aggregate Routes of Exposure to Selected Population Subgroups

Routes and Duration		10% RBC AChE Inhibition	
	Exposure Scenario[a]	PoD[b]	RfD[c] or RfC[c] (PoD/UF of 100)
Acute Oral [µg/kg/day]			
Infant <1	Dietary	600	6.00
Children 1-2	Dietary, Spray-Drift, Aggregate	581	5.81
Children 6-12	Dietary	530	5.30
Females 13-49	Dietary, Spray-Drift	467	4.67
Steady State Oral [µg/kg/day]			
Infant <1	Dietary	101	1.01
Children 1-2	Dietary, Spray-Drift, Aggregate	99	0.99
Children 6-12	Dietary	80	0.80
Females 13-49	Dietary, Spray-Drift	78	0.78
Steady State Dermal [µg/kg/day]			
Children 1-2	Spray-Drift, Aggregate	134250	1342.5
Females 13-49	Spray-Drift	23600	236
Steady State Inhalation [µg/m^3]			
Children 1-2	Spray-Drift, Aggregate	2370	23.7
Females 13-49	Spray-Drift	6150	61.5

a- **Exposure Scenarios**:
Diet: Oral exposure to CPF residues in food and drinking water for the four different population subgroups.
Spray-Drift: Non-occupational/residential bystanders' exposure to CPF due to off-site movement of the product from agricultural applications in California. Females of childbearing age (13-49 years old) and children 1-2 years old have been identified as the potential sensitive population subgroups due to their anticipated high exposures from treated turf and contaminated lawn via dermal contact and inhalation; and for children, mouthing activities such as hand-to-mouth, object-to-mouth, and incidental soil ingestion.
Aggregate: Combined exposures from dietary (food only) and drinking water plus spray drift exposures from inhalation and deposition (i.e., dermal contact for children and adults and mouthing activities for children: object-to-month, hand-to-mouth, and incidental ingestion)

b- **Point of Departure (PoD):** As defined by US EPA (2012), 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. In this Toxic Air Contaminant (TAC) Evaluation, the critical response (PoD) for CPF is defined as 10% RBC AChE inhibition.

c- **Reference Dose (RfD) or reference concentration (RfC):** As defined by US EPA (2012), a RfC or RfD is an estimate of the concentration or dose of a substance (with uncertainty spanning perhaps an order of magnitude) to which a human population can be exposed (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. For CPF, the uncertainty factors (UF) employed are 10 for intraspecies variability based on 10% RBC AChE inhibition and 10 for database uncertainties for neurodevelopmental effects (Total UF = 100): RfD/RfC = (PoD ÷ UF of 100).

No risks were identified from exposures to children and females of childbearing age from dietary sources (food and drinking water) and dermal exposures resulting from spray drift. Potential health risks were identified as: hand-to-mouth exposure in children; inhalation exposure in children and women of childbearing age; and various aggregate exposures from combined media including dietary (food only), drinking water, and deposition and inhalation from spray-drift.

TECHNICAL SUMMARY

Chlorpyrifos (CPF) is a chlorinated organophosphorus (OP) ester used as an insecticide, acaricide, and miticide. The toxicity of CPF is associated with binding and inhibition of the enzyme acetylcholinesterase (AChE) in insects and mammals. CPF requires metabolic activation to CPF-oxon to yield anticholinesterase activity. CPF causes developmental neurotoxicity at exposure levels that do not induce overt toxicity or inhibit cholinesterase (ChE) activity.

The major uses of CPF in California are as an insecticide for nut trees, fruit, vegetable, and grain crops. There are also several registered non-production agricultural uses including uses on golf course turf, industrial sites, greenhouse and nursery production, seed treatments, sod farms, and wood products. Additional uses include cattle ear tags, roach bait (childproof) for use in homes and sewer manholes, and fire ant control in the utility industry. CPF is also used in the public health control of mosquitos. California is the only state that regulates CPF as a restricted use material (http://www.cdpr.ca.gov/docs/legbills/rulepkgs/14-002/final_text.pdf).

CPF was given a "High" priority status by the California Department of Pesticide Regulation (DPR) due to concerns regarding 1) potential neurodevelopmental/ neurobehavioral effects from exposure during vulnerable developmental windows in fetuses, infants, and children, 2) genotoxicity and reproductive toxicity in rats, 3) probable human exposure due to spray drift, 4) possible infant exposure from hand-to-mouth activities, and 5) exposure through food and drinking water. Based on its high priority status, CPF entered the DPR's process of comprehensive human health risk assessment in 2011 (http://www.cdpr.ca.gov/docs/risk/raprocess.pdf and http://www.cdpr.ca.gov/docs/dept/prec/2011/prec_letter_report_52_20110916.pdf).

This risk assessment addresses potential human effects arising from exposure to CPF from food, drinking water, air and skin contact, incidental ingestion contact, as well as aggregate exposures from various combined scenarios.

Chemical Identification and Technical/Product Formulation

CPF (Trade name- Dursban®, Lorsban®; O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate; CAS# 2921-88-2) is a crystalline broad-spectrum insecticide that was first manufactured by Dow AgroSciences LLC in 1965. In the 1990s, CPF was one of the top selling pesticides in the world. Over the last decade, concerns regarding toxicity to the developing nervous system have limited its use.

In December 2000, US EPA reached an agreement to halt the manufacture of chlorpyrifos for nearly all residential uses[1]. Registration was cancelled in March 2001 for indoor residential products except for containerized baits in child resistant packaging. Outdoor residential products

[1] 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

were cancelled except for products specifically for fire ant mound treatment by licensed applicators or mosquito control by public health agencies. All retail sales were stopped in December 2002.

Uses in California

A query of the California Product/Label Database identified 48 products with active registrations in California. Among those, 24 products have labeling language that specifies aerial and/or ground-based application methods. Use fluctuates from year to year. However, the total yearly use of CPF between 2011 and 2015 has ranged from a low of 1.10 million pounds in 2012 to a high of 1.46 million pounds in 2013, with an average application of 1 lb/acre on 0.9 – 1.3 million acres. Almonds received the highest poundage of CPF compared to other crops (range: 192,482 in 2012 to 450,403 lb in 2013).

Illness and Exposure Reports

From 2004-2014, there were 246 associated cases of pesticide exposure stemming from 84 episodes involving chlorpyrifos. The average number of chlorpyrifos episodes per year was 2.9 and the average number of cases was 22.3 per year. The majority of illnesses were due to drift of pesticides (n=163, 66.2%), followed by residue (n=42, 17%). Ingestion accounted for 12 (5%) cases, eight of which resulted from improperly stored pesticides and/or pesticides that were easily accessible by children. Bystanders accounted for 217 (88.6%) of the reported illnesses and most were engaged in routine activities at the time of exposure (n=101, 41%).

Data available from the California Environmental Contaminant Biomonitoring Program (CECBP) gives an indication of background environmental exposure to chlorpyrifos and/or chlorpyrifos-methyl via the measurement of the urinary metabolite 3,5,6-trichloro-2-pyridinol (TCPy) in several study groups. In 112 male and female subjects from California's Central Valley, TCPy was detected in 81% of the urine specimens above the limit of detection (LOD). The geometric mean was 1.23 µg/L. In a group of 101 Orange County, CA firefighters, TCPy was detected in 89% of the samples, with a geometric mean of 1.78 µg/L. In a study conducted at the San Francisco General Hospital, 89 third-trimester maternal urine samples collected from mother-infant pairs had a geometric mean TCPy concentration of 0.52 µg/L (95% CI 0.41- 0.65 µg/L).

TOXICOLOGY PROFILE

The neurotransmitter acetylcholine (ACh) is hydrolyzed by cholinesterase enzymes (ChE), a type of serine hydrolase. AChE hydrolyzes ACh at synaptic clefts in the central nervous system at the neuromuscular or neuro-glandular junctions in the peripheral nervous system and in some non-neuronal cells such as erythrocytes (red blood cells, RBC). When AChE inhibition occurs in nerve and muscles, ACh accumulates and causes unremitting nerve impulses that lead to continuous muscle responses in the peripheral nervous system or neural stimulation in the central nervous system. Butyrylcholinesterases (BuChE/plasma ChE), which represent the majority of the ACh-hydrolyzing activity in human plasma, are also inhibited by CPF, though the toxicological consequences of this inhibition are not fully understood.

The active CPF metabolite, CPF-oxon, inhibits AChE by binding at the active site of the enzyme. CPF-oxon also inhibits the BuChE enzyme. AChE inhibition in red blood cells is commonly used as a surrogate of the inhibition in target tissues.

Metabolism

The estimated oral absorption of CPF is 70-99% in rats and humans. Dermal and inhalation absorption is mostly indicated from inhibition of ChE activities and urinary recovery of metabolites. In animals and humans, CPF is extensively metabolized by the liver cytochrome P450 enzymes (CYP1A2, 2B6, 2C19, 3A4, 3A5, and 3A7). Oxidative desulfuration results in CPF-oxon. Dearylation of CPF and CPF-oxon by CYP produces TCPy and diethyl thiophosphate (DETP). Hydrolysis of the CPF-oxon by B-esterases (BuChE and carboxylesterase, CES) and A-esterases (paraoxonases, PON1) detoxify CPF-oxon to the urinary metabolite TCPy, which is used as a biomarker for CPF exposure. CPF is detected in rat and human milk. In rats, transplacental transfer to the fetus is evidenced by ChE inhibition in fetal plasma and brain and by the presence of CPF in fetal liver, brain, placenta, umbilical cord, and amniotic fluid.

Acute and Short-Term Toxicity

CPF is classified by US EPA as a moderate oral toxicant (Category II). The acute oral LD_{50} is 32 mg/kg for hens and 82 to 504 mg/kg for rats, mice, and guinea pigs. The oral LD_{50} for CPF-oxon is > 100 mg/kg in male rats and 300 mg/kg in female rats. The dermal LD_{50} in rats is 202 mg/kg/d. The 4-hour inhalation LC_{50} in rats is > 2 mg/L. CPF is a Category IV skin and eye irritant, causing slight conjunctival and dermal irritation. Human deaths are reported due to accidental exposure or intentional ingestion. CPF doses > 300 mg/kg in humans have resulted in unconsciousness, convulsions, cyanosis, and uncontrolled urination.

The main target of CPF toxicity after short-term excessive oral exposure (not those expected from typical ambient, real-world exposure) is the nervous system of adult and developing organisms. Cholinergic syndromes resulting from the overstimulation of the muscarinic and nicotinic ACh receptors include hypersalivation, respiratory distress, miosis, muscular twitches, tremors, ataxia, diarrhea, and vomiting. Other effects include hematological and liver enzyme changes, chromodactyorrhea, tachycardia, renal effects, hypothermia, and body weight decreases. No delayed neuropathy was observed in hens.

As with other OPs, the critical no-observed effect levels (NOELs) for CPF are typically based on RBC or brain AChE inhibition, for which robust data in animals and humans are available. A Benchmark Dose (BMD) analysis performed by US EPA in 2011 calculated a BMDL (lower bound of BMD) of 0.36 mg/kg/d based on 10% RBC ChE inhibition in rat pups on postnatal day (PND) 11 after a single oral exposure. For acute CPF-oxon exposure, the similarly determined BMDL is 0.08 mg kg/day. In 2014, US EPA used a physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) model to estimate the critical toxicological points of departure (PoDs) for CPF. These PoDs are human equivalent doses based on 10% inhibition of the RBC AChE activity after an acute (single day, 24 hr) or subchronic (steady-state, 21-days) exposure (Summary Table 1). The acute PoDs for children and females of childbearing age were 0.457-0.6 mg/kg/d and the steady state PoDs were 0.078-0.1 mg/kg/d.

Chronic Toxicity

Effects reported in workers chronically exposed to CPF included impaired memory, disorientation, speech difficulties, nausea, and weakness. The most sensitive effects observed after chronic dietary exposure to CPF in rats and mice were ChE inhibition, neurological signs, developmental neurotoxicity, and neurobehavioral effects. At higher doses, there was evidence of increased adrenal gland, brain and heart weight in rats, increased liver weight, and hepatocyte vacuolation in dogs and mice, and ocular opacity and hair loss in mice. In 2011, US EPA established a chronic BMDL of 0.03 mg/kg/d based on 10% RBC AChE inhibition in PND 11 male rats after 11 days of oral exposures.

Reproductive and Developmental Toxicity

The available two-generation reproductive toxicity studies in rats indicate that CPF is not teratogenic and does not adversely affect reproduction. In prenatal developmental toxicity studies in rats and mice, fetal growth retardation and developmental delays were observed in the presence of maternal toxicity.

Developmental Neurotoxicity

CPF may cause developmental neurotoxicity in rats and mice at doses that elicit minimal or no fetal brain AChE inhibition. Three major prospective cohort studies in humans evaluated pre- and post-natal pesticide exposure in mother-infant pairs and birth and developmental outcomes in neonates, infants, and children. One study from Columbia University in New York City Columbia Center for Children's Environmental Health (CCCEH) focused on CPF levels in the umbilical cord and maternal plasma as a direct biomarker for CPF *in utero* fetal exposure. The other two studies from Mount Sinai Hospital in New York City and from the University of California at Berkeley measured TCPy (a metabolite of CPF and CPF methyl) and non-specific OP metabolites in maternal urine. Collectively, the results from these studies have shown associations of indoor and outdoor exposure to CPF during pregnancy with adverse neurodevelopmental outcomes in children through age 11 years, including changes in brain morphology, delays in cognitive and motor functions, and problems with attention, and tremors.

Genotoxicity

CPF is negative for gene mutation (*Salmonella typhimurium*, *Escherichia coli*, Chinese hamster ovary cell) and chromosomal aberrations (rat lymphocytes, mouse bone marrow micronucleus). Assays for DNA damage were negative in mammalian cells, but positive in yeast and bacteria.

Carcinogenicity

CPF did not cause tumors in chronic oral studies with rats and mice. Currently CPF is not listed as a carcinogen (http://monographs.iarc.fr/ENG/Classification/List_of_Classifications.pdf; http://monographs.iarc.fr/ENG/Classification/index.php) by the International Agency for Research on Cancer (IARC), the US EPA Toxics Release Inventory Criteria (TRI), or California Proposition 65. The US EPA Office of Pesticide Programs states, "Chlorpyrifos is not likely to be carcinogenic to humans, based on the lack of evidence of carcinogenicity in studies in rats and

mice and the absence of a mutagenicity concern" (US EPA, 2011, Preliminary Human Health Risk Assessment for Chlorpyrifos, page 159.)

Immunotoxicity

Studies in rodents, cats, and dogs indicate that at doses causing ChE inhibition, CPF did not alter immune system function.

ToxCast™ Profiles and Tox21 HTS Profiles

The Toxicity ForeCaster (ToxCast™) and Tox21 high-throughput screening assays (HTS) were examined for indications of pathway disruptions that could lead to toxic effects. Zebrafish is a promising test model to examine the potential CPF neurobehavioral effects and compare active concentrations to those inhibiting ChE activity. Abnormal behaviors (increased "fish at rest," decreased swim speed, decrease in fish with a preference for being on the side or on the edge of their swim lane) occur at CPF levels 10-fold lower than those inhibiting AChE. This provides support for the use of UF of 10 to account for the potential neurodevelopmental effects.

ToxCast and Tox21 provide indications of CPF pathway disruptions in cell adhesion, cell cycle, and cell morphology assays. CPF is also a positive hit for molecular targets that regulate 1) induction and inhibition of CYP enzymes, 2) hormone levels in the brain, 3) endocrine receptor binding, and 4) inhibition of steroidogenesis. However, it is unclear if these impacted pathways are potential key noncholinergic molecular events responsible for the observed CPF neurodevelopmental toxicity *in vivo*.

RISK ASSESSMENT

A comprehensive human health risk assessment was conducted for 4 sentinel subgroups of the general population: infants (<1 year old), children 1-2 years old, children 6-12 years old, and females of childbearing age (13-49 years old).

Hazard Identification

The critical NOELs for evaluating oral, dermal, and inhalation exposure to CPF from diet and spray drift were toxicological PoDs based on inhibition of the RBC AChE activity. HHA used the PoDs from the US EPA 2014 Revised Human Health Risk Assessment as a starting point for this risk assessment. The PoDs are PBPK-PD model-derived human equivalent doses based on 10% inhibition of the RBC AChE activity after an acute (single day, 24 hr) or steady-state (21-days) exposure of CPF in humans (Summary Table 1). The PBPK-PD model includes parameters that account for human-specific physiology and metabolism for all age groups, as well as multi-route variations in RBC AChE inhibition that account for variation in the sensitivity within the human population (infants, children, youths, and non-pregnant adults).

Summary of Critical NOELs

Summary Table 1. Critical NOELs (PoDs) for CPF and CPF-Oxon

Exposure Route[a]	PBPK-PD PoDs (US EPA, 2014a)							
	Infants < 1 yr old		Children 1-2 yrs old		Child 6-12 yrs old		Females 13-49 yrs old	
	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]
Dietary (food only) and Drinking Water Exposures								
Drinking H_2O (oxon ppb)	1,183	217	3,004	548	7,700	1,358	5,285	932
Food (mg/kg/d)	0.600	0.103	0.581	0.099	0.530	0.090	0.467	0.078
Non-Dietary Exposures								
Incidental Oral (mg/kg/d)	--	--	--	0.101	--	--	--	--
Dermal (mg/kg/d)	--	--	--	134.25	--	--	--	23.60
Inhalation (mg/m^3)	--	--	--	2.37	--	--	--	6.15

Abbreviation: PoD, point-of-departure; CPF, chlorpyrifos; CPF-Oxon, chlorpyrifos-oxon; PBPK/PD, physiological-based pharmacokinetic/pharmacodynamic model; SS, steady state

[a] PoDs are human equivalent doses derived from PBPK/PD model based on 10% inhibition of the RBC AChE activity after an acute (single day, 24 hr) or steady-state (21-day) exposure to CPF in humans (US EPA, 2014a). PoD from parent compound CPF was used for all exposure routes except for drinking water where the PoD from CPF-oxon was used.

[b] This assessment used SS oral (non-dietary), dermal, and inhalation PoDs to estimate the risk from spray drift and aggregate exposures.

[c] Acute PoDs for CPF-oxon in ppb (µg/L) were converted into internal doses (mg/kg/d) using default drinking water consumption and body weight values .

[d] Steady-state dermal PoDs for CPF were developed assuming exposure duration of 1.5 hours per day for 21 days (US EPA, 2014a).

[e] Steady-state inhalation PoDs were developed assuming exposure duration of 1 hour per day for 21 days (US EPA, 2014a).

EXPOSURE ASSESSMENT

Spray Drift Residue Exposure Estimates

Exposure associated with spray drift near an application site was evaluated for two of the sentinel population subgroups: children 1-2 years old and females of childbearing age (13-49 years old). In this exposure assessment, females 13-49 years old are a primary focus because of their potential increase in susceptibility to the toxicological effects of CPF during pregnancy. For children 1-2 years old, the US EPA Residential SOP (Addenda 1: Consideration of Spray Drift), indicates that children at this lifestage exhibit the highest exposure potential to pesticide contaminated lawn from spray drift due to dermal contact and different mouthing activities such as hand-to-mouth, object-to-mouth, and incidental soil ingestion. The SOP assumed that the duration of exposure for females 13-49 years old and children 1-2 years old near the application sites would be 1.5 hours.

Aerial Applications

Single application horizontal deposition exposure (in µg/kg/day) and inhalation exposure estimates (as 1 hour time-weighted average air concentrations in mg/m^3) of CPF were considered for two subpopulations: females 13-49 years old and children 1-2 years old and three application rates for two types of aircraft: fixed-wing (AT802A airplane) and rotary (Bell 205 helicopters). Increases in CPF application rate resulted in a corresponding increase in the horizontal deposition exposure estimates (regardless of exposure route) at different distances downwind from the edge of the treated field. Akin to the deposition estimates, the inhalation exposure estimates increase with the application rates.

For the aerial application, some CPF-containing products specify a minimum spray volume of not less than 2 gallons per acre. However, there appears to be no maximum spray volume specified. To evaluate the effect of spray volume on the horizontal deposition and inhalation exposure estimates, an additional AGricultural DISPersion (AGDISP) simulation was performed. As distance from the application edge increases, for a given application rate, both the horizontal deposition exposure estimates and the estimated 1 hour time-weighted average air concentrations increase with the spray volume.

Ground-Based Applications

Horizontal deposition exposure estimates (in µg/kg/day) of CPF were evaluated for the same two 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 CPF for children 1-2 years old after ground boom or airblast application showed that exposure increases with application rates of CPF. 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.

Dietary Exposure Assessment- Food and Drinking Water

CPF is used on a wide variety of food crops in California. Based on the most recent five years of use data (2011-2015), the top agricultural uses in the state were almond, alfalfa, walnut, orange, and cotton.

In 2014, US EPA conducted highly refined probabilistic acute and steady-state (21-day) dietary (food-only) exposure assessments of CPF. They evaluated the exposure to CPF from drinking water by estimating concentrations of CPF-oxon in surface and ground water (Estimated Drinking Water Concentrations, EDWC) and comparing the values to target concentrations expressed as DWLOC (Drinking Water Level of Comparison).

No new uses for CPF have been introduced since December 2014. Therefore, it was not necessary to conduct an independent dietary exposure assessment. Instead, HHA utilized the 2014 US EPA food-only exposure estimates to evaluate the risk from CPF exposure from food. HHA conducted an independent drinking water exposure assessment employing residue data from surface water in California and PDP monitoring data for drinking water in California.

Dietary (food-only) Exposure Assessment

Acute and subchronic (21-day steady-state) food-only exposures were calculated for four sentinel subpopulations identified in the US EPA risk assessment: infants (< 1 year old), children 1-2 years old, children 6-12 years old, and females of childbearing age (13-49 years

old). Children 1-2 years old were identified as the highest exposed population subgroup. At the 99.9th percentile, acute exposure was estimated to be 0.000423 mg/kg/d and steady-state exposure was estimated at 0.000242 mg/kg/d.

Drinking Water Exposure Assessment

CPF is rapidly oxidized to the oxon during the chlorination process. In this assessment, HHA assumed that 100% of CPF is converted to CPF-oxon during water treatment. HHA estimated drinking water probabilistic exposures using 1) Pesticide Data Program (PDP) drinking water residue data for CPF or 2) CPF residue data from the DPR Environmental Monitoring Branch (EMON) surface and ground water databases, and 3) drinking water consumption records in the Dietary Exposure Evaluation Model-Food Commodity Ingredient Database (DEEM-FCID™, version 2.036) for acute exposure. The analyses showed that exposures from residues in surface water in California could be as much as 4-fold higher than exposures based on the PDP CA-specific drinking water monitoring data.

Analysis of Drinking Water Exposure Using PDP Residue Data

PDP data from 2001 to 2013 were used in this analysis. A total of 706 post-treatment samples from municipal water treatment plants were analyzed for CPF-oxon. No residues were detected. Exposure to CPF-oxon in drinking water was estimated by assuming that each sample contained CPF-oxon at concentrations equivalent to the analytical limit of detection (LOD) for CPF. The 99.9th percentile exposure for all infants, the most highly exposed subpopulation, was 0.000108 mg/kg.

Analysis of Drinking Water Exposure Using DPR Surface and Ground Water Residue Data

Pesticide residues in water are monitored by the DPR surface and ground water programs. These programs are biased toward capturing higher concentrations that coincide with agricultural runoff, storm events, and pesticide use and applications. The DPR monitoring programs detected high residue levels in samples collected from various water sources including irrigation ponds, sloughs, and agricultural drains. DPR residue databases also contain analytical results reported by other California state and local agencies.

Between 2005 and 2014, a total of 7154 surface water samples were analyzed for CPF. The range of detected residues was 0.000572 to 3.7 ppb. For ground water, 2055 samples were analyzed from 2004 to 2013. Only two samples had detectible residues (0.006 and 0.008 ppb). Acute exposure to CPF-oxon in drinking water was estimated by conducting a probabilistic analysis of either the detected CPF residue in surface water or the detection limit (in the case of non-detects) together with all reported individual water consumption records for each subpopulation. The 99th percentile exposures for the most highly exposed subpopulation, all infants <1 years old, were 0.000419 mg/kg (surface water) and 0.000222 mg/kg (ground water).

RISK CHARACTERIZATION

The critical NOELs (PoDs) for characterizing the risk from exposure to CPF were PBPK-PD-estimated human equivalent doses. Risks were calculated as margin of exposure (MOE), a ratio of the NOEL to the human exposure level. A target MOE of 100 is generally considered protective against the CPF toxicity. This target takes into account uncertainty factors of 1 for interspecies sensitivity, 10 for intraspecies variability, and 10 for potential neurodevelopmental effects. When exposure occurs by more than one route, route-specific NOELs are used and combined MOE for all routes can be calculated.

Bystander Spray Drift MOEs

Spray drift exposure is of short-term duration (1 – 1.5 hours). Typically, acute PoDs would be used to estimate the risk associated with the short-term exposure. However, using acute PoDs may underestimate risks to individuals residing in areas of high CPF use because these values do not account for the reduced RBC AChE activities in such populations as a result of constant exposure that certainly occurs in high-CPF use areas. Indeed, data on RBC AChE levels in children residing in such areas show that their enzyme activities are decreased by about 30% compared to children who live in non- or low-use agricultural areas. Therefore, when evaluating the risk from a short term exposure in the presence of concurrent background exposures for populations in areas of high CPF use, we considered three critical factors: 1) AChE inhibition is cumulative in nature; 2) Studies in humans show that while CPF inhibits RBC AChE activity after a single dose, full recovery of enzyme activity is not attained even after 10 days; and, 3) AChE inhibition in repeated dosing studies in animals reaches steady state levels after ~2-3 weeks of exposure. In light of the reduced levels of AChE activity due to background exposure in high-use areas and the slow recovery of enzyme activity after CPF exposure, HHA concluded that the effect produced from short term drift exposures would be best characterized by the PoD derived from repeated (21-day) dosing.

MOEs for spray drift were estimated for females 13-49 years old and children 1-2 years old that were exposed at 10-1000 feet from CPF treated fields. Different exposure routes associated with spray drift were evaluated: 1) dermal exposure through skin contact; 2) inhalation exposure; and, 3) oral non-dietary exposure due to mouthing activities of young children such as hand-to-mouth, object-to-mouth, and incidental soil ingestion. The combined exposures included different portals of entry (dermal, oral, and inhalation) and exposure durations (1-1.5 hours near the application field and 1 day of food and drinking water consumption). Consequently, route-specific MOEs were used to characterize the risks associated with each route.

Females 13-49 years: The MOEs for dermal exposure near the application site were greater than the target of 100 for all evaluated scenarios: aerial application with the fixed-winged and rotor-wing aircrafts at the application rates of 1, 2, or 2.3 lb a.i./acre; and ground boom and airblast at the application rates of 1, 2, 4, or 6 lb a.i./acre. However, the MOEs for inhalation exposure near the application site were less than the target of 100 for some application scenarios: aerial application with the fixed-winged and rotor-wing aircrafts up to 10 ft for application rates of 2 or 2.3 lb a.i./acre; ground boom or airblast up to 50 ft for application rates at 4 or 6 lb a.i./acre.

Children 1-2 years: All MOEs for dermal or oral exposures (object-to-mouth and incidental soil ingestion) were greater than the target of 100 for both aerial and ground-based applications. The oral MOEs from hand-to-mouth exposure were greater than 100 at all distances using aerial or airblast equipment at an application rate of 1 lb a.i./acre. However, the oral MOEs from hand-to-mouth exposure were lower than 100 up to 50 feet from the aerial application starting at 2 lb a.i./acre and up to 25 feet of the airblast application at 6 lb a.i./acre. The inhalation MOEs were lower than the target of 100 for children up to 50 feet at 1 lb a.i./acre, 100 feet at 2 lb a.i./acre, and 250 feet at 2.3 lb a.i./acre from the edge of a treated field after applying CPF with aerial equipment. For ground boom and airblast the inhalation MOEs were less than the target of 100 for children up to 75 ft for 1 lb a.i./acre, 200 ft for 2 lb a.i./acre and 250 ft for 4 and 6 lb a.i./acre.

Dietary (food only) Exposure MOEs

At the 99.9th percentile, the acute dietary MOEs from exposure to CPF residues in food ranged from 1374 to 3127 for the four at risk subpopulations. At the 99.9th percentile, the steady state MOEs for these subpopulations ranged from 409 to 1040. All acute and steady state MOEs were greater than the target of 100.

Drinking Water Exposure MOEs

The acute MOEs for exposure to CPF-oxon in drinking water for the four at-risk subpopulations were based on drinking water residues from PDP or from the DPR surface and ground water programs. At the 99.9th percentile, the MOEs were highest for PDP (1571-3970) and lowest for the DPR surface water (405-1299). All MOEs for acute water-only exposure were greater than the target of 100.

Aggregate Exposure MOEs

For aggregate exposures, it was assumed that a child 1-2 years old would be exposed at 10-1000 feet from the CPF application site potentially through inhalation, skin contact with residues (spray drift deposition), ingestion of residues by object-to-mouth, hand-to-mouth, and incidental soil ingestion (oral exposure), and consumption of food and drinking water. An aggregate MOE approach was used because of different exposure routes and durations.

The PoD values used for the risk characterization of aggregate exposures to children 1-2 years old are shown in Summary Table 1. For the combined deposition, the risk was calculated using the steady state (21-day) dermal, inhalation, and oral PoDs for CPF and the short-term (1-1.5 hours) dermal, inhalation, and non-dietary oral exposures. The acute dietary risk from food-only or drinking water probabilistic 99.9th percentile exposures was calculated using the acute oral PoD for CPF and the acute oral PoD for CPF-oxon, respectively. Drinking water exposures were based on residues from PDP or the DPR surface water monitoring program.

The acute aggregate MOEs were estimated for all routes, including combined deposition:

$$\text{Aggregate MOE} = \frac{1}{\frac{1}{MOE_{CD}} + \frac{1}{MOE_{I}} + \frac{1}{MOE_{D}} + \frac{1}{MOE_{DW\ (PDP\ or\ EMON)}}}.$$

Abbreviations: CD [dermal + oral (object-to-mouth + hand-to-mouth + soil deposition)], inhalation (I), and oral from dietary sources (D: food only) and drinking water (DW).

The aggregate MOEs for a number of combined scenarios were below the target of 100 (Summary Table 2). The inhalation exposures made a substantial contribution to the aggregate exposure. Consequently, the combined MOEs were significantly reduced when inhalation exposures were added to the dermal, non-dietary oral, and dietary exposures. Therefore, inhalation exposure to CPF near the application site was the critical driver of the aggregate MOEs below the target value of 100 for children 1-2 years old (Summary Table 2).

RISK APPRAISAL

The main uncertainties associated with CPF toxicity and the use of 10% RBC AChE inhibition as toxicological PoDs were:

(i) Selection of 10% RBC AChE inhibition as the critical toxicity endpoint was intended to protect human populations from impacts on other endpoints that were not easily measured. However, collective results from epidemiology and animal toxicology studies indicate that CPF may be associated with neurodevelopmental and neurobehavioral effects at concentrations below those that cause AChE inhibition.

The main uncertainties in the exposure assessment were:

(i) Default physiological parameters and standard modeling and exposure computational methodologies were used to estimate bystanders' exposures (i.e., children 1-2 years old and adults only).

(ii) Illegal residues measured in fresh produce in California were not included in the dietary exposure assessment. PDP frequently detected CPF residues on crops that lack tolerances. In California, the DPR's Pesticide Residue Monitoring Program (CPRMP) monitors fresh produce collected throughout the channels of trade, including wholesale and retail outlets, distribution centers, and farmers markets (http://www.cdpr.ca.gov/docs/enforce/residue/rsmonmnu.htm). From 2015 to 2017, CPRMP detected CPF in 2547 samples of fresh produce, of which 269 (11%) were illegal. A high proportion of illegal detections were on litchi, orange, oriental pear, cactus and tangelo. Most of these foods were imported. Certain population ethnic subgroups (e.g., Hispanic and Asian) in California have higher consumption of these foods. HHA evaluations of these cases concluded that 23 were of potential health risk to consumers. HHA does not evaluate illegal residues on agricultural commodities in its dietary exposure assessments. Such residues come under the purview of DPR's Enforcement Branch, which has the authority to remove affected produce from channels of trade.

(iii) HHA estimated the exposure to CPF in drinking water using residue data from PDP or DPR surface and ground water monitoring programs. The analyses showed that exposures from residues in surface water in California could be up to 4-fold higher than exposures based on the PDP California-specific drinking water monitoring data, although those surface water sources are not necessarily drinking water sources. The use of PDP data may

lead to an underestimation of the drinking water exposure because PDP is not designed to detect peak concentrations of CPF-oxon in drinking water and the estimated exposures were based entirely on LODs. In contrast, drinking water exposure based on residues from the DPR surface and ground water programs would likely represent the "high-end" of the potential exposure, because these programs are biased toward capturing higher concentrations coinciding with runoff timing, storm events, and timing of pesticide use and applications. In addition, DPR monitoring programs detected high residue levels in samples collected from various water sources, including irrigation ponds, sloughs, and agricultural drains that may not be used for drinking water. Therefore, the drinking water exposure estimates in this risk assessment are considered highly conservative.

The main uncertainties in the risk characterization were:

(i) A default assumption of 10-fold was used due to database uncertainties in the PBPK-PD model. Predictions for variation in human sensitivity could not be used to reduce the default 10x intraspecies uncertainty factor because the model could not fully account for physiological, anatomical, and biochemical changes during pregnancy. Consequently, a default uncertainty factor of 10 instead of the pregnancy version of the PBPK/PD model was used to account for the sensitivity within the human population with respect to RBC AChE inhibition.

(ii) A default uncertainty factor of 10 was used to account for potentially more sensitive neurodevelopmental effects than AChE inhibition, the critical endpoint used to characterize the risk from CPF exposure. Effects on cognition, motor control and social behavior have been consistently reported in the CPF epidemiology and animal toxicology studies. However, these studies were not sufficient to derive critical points of departure for neurodevelopmental effects due to uncertainties associated with dose-response characteristics and exposure duration. Moreover, most animal studies were conducted with doses that also produced AChE inhibition at some time during the exposure. The document includes evidence for CPF-induced behavioral effects in young rats that may occur at doses up to 10-fold lower than the threshold established for RBC AChE inhibition, though as noted, precise quantification was not possible.

(iii) For spray drift, the risk from short-term (1-1.5 hour) dermal, inhalation, and non-dietary oral exposures was calculated using the steady-state (21-day) dermal, inhalation, and oral PoDs for CPF. Assuming the cumulative inhibitory effect of CPF on RBC AChE and the concurrent background exposure, acute PoDs may not be sufficient for characterizing the AChE inhibition from spray drift.

(iv) Drinking water exposure for children 1-2 years old was used for an aggregate MOE calculations even though infants <1 year old received the highest exposure to CPF-oxon in drinking water. This was done because the 99th percentile drinking water exposure for children 1-2 years old matches the population subgroup evaluated for exposure to food and spray drift. Had the drinking water exposure estimates for infants <1 year old been used, the drinking water MOEs would be 2-fold lower.

CONCLUSIONS

The health risk assessment of CPF was conducted for 4 sentinel subpopulations: infants (<1 year old), children 1-2 years old, children 6-12 years old, and females of childbearing age (13-49 years old).

Single-route exposure scenarios were evaluated for children 1-2 years old and females of childbearing age under short-term conditions associated with spray drift near the application site: dermal exposure through skin contact, inhalation exposure, and oral non-dietary exposure due to mouthing activities of young children (hand-to-mouth, object-to-mouth, and incidental soil ingestion). Dietary exposures from food for acute (1 day) or steady state (21-days) durations and acute (1 day) drinking water exposures were also calculated. Aggregate exposures involving multiple routes were calculated for females of childbearing age and children 1-2 years old at 10-1000 feet from the CPF application site. These routes included inhalation, skin contact with residues (horizontal deposition and aerosols associated with spray drift), ingestion of residues by object-to-mouth, hand-to-mouth and incidental soil ingestion (oral non-dietary exposure), and consumption of food and drinking water (oral, dietary exposure).

The critical NOELs or toxicological points of departure (PoDs) for CPF were PBPK-PD estimated human equivalent doses based on 10% RBC AChE inhibition. A MOE of 100 was considered protective against the CPF toxicity in humans. The target of 100 includes uncertainty factors of 1 for inter-species sensitivity, 10 for intra-species variability, and 10 for potential neurodevelopmental effects.

Spray Drift Exposure:

Females 13-49 years old: The MOEs for dermal and inhalation exposure near the application site were less than the target of 100 for some application scenarios: aerial application with the fixed-winged and rotor-wing aircrafts up to 10 ft for application rates of 2 or 2.3 lb a.i./acre; ground boom or airblast up to 50 ft for application rates at 4 or 6 lb a.i./acre.

Children 1-2 years old: All MOEs for dermal and oral exposures (object-to-mouth and incidental soil ingestion) were greater than the target of 100 for both air and ground-based applications. The oral MOEs from hand-to-mouth exposure were greater than 100 at all distances using aerial or airblast equipment at an application rate of 1 lb a.i./acre. However, the oral MOEs from hand-to-mouth exposure were lower than 100 up to 50 feet from the aerial application starting at 2 lb a.i./acre and up to 25 feet of the airblast application at 6 lb a.i./acre. The inhalation MOEs were lower than the target of 100 for children up to 50 feet at 1 lb a.i./acre, 100 feet at 2 lb a.i./acre, and 250 feet at 2.3 lb a.i./acre from the edge of a treated field after applying CPF with aerial equipment. For ground boom and airblast, the inhalation MOEs were less than the target of 100 for children up to 75 ft for 1 lb a.i./acre, 200 ft for 2 lb a.i./acre and 250 ft for 4 and 6 lb a.i./acre.

Dietary Exposure:

Food-only exposure: At the 99.9^{th} percentile, the acute dietary MOEs from exposure to CPF residues in food ranged from 1374 to 3127 for the four evaluated sentinel population subgroups.

At the 99.9th percentile, the subchronic (21-day, steady state) MOEs for these subpopulations ranged from 409 to 1040. All acute and steady state MOEs were greater than the target of 100.

Drinking water exposure: The acute MOEs for exposure to CPF-oxon in drinking water for the four sentinel populations were based on drinking water residues from PDP or from the DPR's surface and ground water monitoring programs. At the 99.9th percentile, the MOEs were highest for PDP (1571 – 3970) and lowest for the DPR surface water (405 – 1299). All MOEs for acute water-only exposure were greater than the target of 100.

Aggregate Exposure: Dietary (food only), drinking water (PDP or DPR surface water) and spray drift

Children 1-2 year old: The acute aggregate MOEs were estimated for all routes, including combined deposition. For the combined deposition, the risk was calculated using the steady state (21-day) dermal, inhalation, and oral PoDs for CPF and the short-term (1.5 h) dermal, inhalation, and non-dietary oral exposures (Summary Table 1). The acute dietary risk at 99th percentile exposures was calculated using the acute oral PoD for CPF (food only) and the acute oral PoD for CPF-oxon (drinking water only), respectively. The drinking water exposures were based on residues from PDP or the DPR surface water monitoring program.

$$\text{Aggregate MOE} = \frac{1}{\frac{1}{MOE_{CD}} + \frac{1}{MOE_{I}} + \frac{1}{MOE_{D}} + \frac{1}{MOE_{DW\ (PDP\ or\ EMON)}}}.$$

CD [dermal + oral (object-to-mouth + hand-to-mouth + soil deposition)], inhalation (I), and oral from dietary sources (D: food only) and drinking water (DW). CPF-oxon residues in drinking water were from PDP or from DPR's surface water monitoring database.

The aggregate MOEs for a number of combined scenarios were below the target of 100 (Summary Table 2). The air component contributed up to 95% to the aggregate exposure. Consequently, the aggregate MOEs were significantly reduced when the inhalation MOE was added to the dermal, non-dietary oral, and dietary MOEs. In conclusion, the exposure to aerosols in the air near application sites was identified as the main driver when the aggregate MOEs fell below the target value of 100 for children 1-2 years old.

Summary Table 2. Aggregate MOEs for Children (1-2 years old) at Various Distances Downwind from Fields Treated with CPF by Fixed Wing Aircraft or Helicopter

Application Scenario	Appl. Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distances Downwind from the Treated Fields						
				10 feet	25 feet	50 feet	100 feet	250 feet	500 feet	1000 feet
Aircraft or Helicopter (Children 1-2 years old)										
AT802A Fixed Wing Aircraft	2	CD[a]	1	127	149	190	282	541	907	1701
			2	63	75	95	143	285	523	1331
			2.3	55	65	83	124	249	469	1210
		CD + I[b]	1	47	53	61	78	116	166	300
			2	26	29	35	46	74	120	264
			2.3	23	27	32	42	69	113	252
		CD + I + D[c]	1	45	51	58	74	107	148	246
			2	25	29	34	44	70	110	221
			2.3	23	26	31	41	65	105	213
		CD + I + D + DW-PDP[d]	1	45	51	58	74	106	147	244
			2	25	29	34	44	70	110	220

			2.3	23	26	31	41	65	104	211
		CD + I + D + DW-EMON[d]	1	43	48	55	68	95	127	193
			2	25	28	32	42	65	98	178
			2.3	22	25	30	39	61	94	172
Bell 205 Helicopter	2	CD	1	100	158	258	424	664	1118	2289
			2	50	78	126	203	367	716	1633
			2.3	43	68	110	176	325	645	1500
		CD + I	1	37	49	65	86	126	192	347
			2	20	27	37	51	85	145	287
			2.3	18	25	34	48	80	140	280
		CD + I + D	1	36	47	62	81	115	169	277
			2	19	26	36	49	80	131	238
			2.3	18	24	33	46	76	127	233
		CD + I + D + DW-PDP	1	36	47	62	81	115	168	274
			2	19	26	36	49	80	131	236
			2.3	18	24	33	46	76	126	231
		CD + I + D + DW-EMON	1	34	45	58	74	102	142	212
			2	19	26	34	47	73	115	188
			2.3	17	24	32	44	70	111	185
AT802A Fixed Wing Aircraft	15	CD	1	147	174	217	325	633	1021	1368
			2	70	83	103	152	288	452	622
			2.3	61	72	89	131	248	390	538
		CD + I	1	39	43	47	56	73	89	115
			2	22	24	27	32	43	55	75
			2.3	19	21	24	29	39	50	69
		CD + I + D	1	38	42	46	54	69	84	106
			2	21	24	26	32	42	53	71
			2.3	19	21	23	28	38	48	66
		CD + I + D + DW-PDP	1	38	42	46	54	69	83	105
			2	21	24	26	31	42	52	71
			2.3	19	21	23	28	38	48	66
		CD + I + D + DW-EMON	1	37	40	44	51	64	77	95
			2	21	23	25	30	40	50	66
			2.3	19	21	23	28	36	46	61
Bell 205 Helicopter	15	CD	1	107	175	301	519	747	996	1521
			2	52	84	141	238	340	478	790
			2.3	45	72	121	204	294	419	692
		CD + I	1	26	33	40	48	59	76	109
			2	17	21	27	33	42	56	84
			2.3	15	19	24	30	39	52	78
		CD + I + D	1	26	32	39	46	57	72	101
			2	16	21	26	33	41	54	79
			2.3	15	19	24	29	38	50	74
		CD + I + D + DW-PDP	1	26	32	39	46	57	72	100
			2	16	21	26	32	41	54	79
			2.3	15	19	24	29	38	50	74
		CD + I + D + DW-EMON	1	25	31	37	44	54	67	91
			2	16	21	26	31	39	51	73
			2.3	14	18	23	29	36	47	68

Source: US EPA (2014a) Dermal PoD-Steady-state = 134.25 mg/kg/d; For calculations, Dermal Absorption (0-1) = 1; Oral PoD Steady-state: 0.099 mg/kg/d. Target MOE = 100

[a] Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion)

[b] Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion; inhalation (I))

[c] Combined Deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d).

[d] Combined Deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON); inhalation PoD = 2.37 mg/m^3

I. INTRODUCTION

This Risk Characterization Document addresses potential human exposures from the use of chlorpyrifos (CPF) in California as an active ingredient (AI) in insecticide formulations for nut trees, fruit, vegetable, and grain crops, as well as for non-food crop uses (e.g., golf course turf, industrial sites, greenhouse and nursery production, sod farms, and wood products) for which there are tolerances. CPF was given a "High" priority status by DPR due to concerns regarding 1) potential neurodevelopmental/ neurobehavioral effects from exposures during vulnerable developmental windows in fetuses, infants, and children, 2) genotoxicity and reproductive toxicity in rats, 3) probable human exposure due to spray drift, 4) possible infant exposure from hand-to-mouth activities, and 5) exposure through food and drinking water in California. Based on its "High" priority status, in 2011 CPF entered the DPR's process of comprehensive human health risk assessment (http://www.cdpr.ca.gov/docs/risk/raprocess.pdf and http://www.cdpr.ca.gov/docs/dept/prec/2011/prec_letter_report_52_20110916.pdf).

An assessment of the relevance of the Physiologically-Based Pharmacokinetic-Pharmacodynamic (PBPK-PD) model utilized by US EPA (2014a) for California-specific exposure scenarios was performed. These data were compiled and evaluated in order to characterize risk from CPF in California.

I.A. Scope

This risk assessment focuses only on effects reported after exposure to CPF. The critical endpoint used throughout the risk characterization is acetylcholinesterase inhibition.

I.B. Regulatory Status

I.B.1. United States Environmental Protection Agency

Regulatory History for Chlorpyrifos:

1965: CPF was registered for residential use in 1965 as a crack and crevice treatment for ants, cockroaches and termites.

1997: The CPF technical registrants agreed to eliminate and phase out residential use due to US EPA concerns for effects to children and other sensitive subpopulations.

2000: All indoor residential CPF use as well as use for termite control in schools, hospitals and nursing homes was discontinued.

2004: CPF for termite control in new construction was discontinued.

2006: The US EPA CPF Reregistration Eligibility Decision (RED) was completed. Critical endpoints were established based on 10% RBC and plasma ChEI in adult rats.

2007-2008: Dow AgroSciences wrote commentaries rebutting fetal growth and developmental findings.

2007: National Resources Defense Council (NRDC) petitioned US EPA to ban CPF for all uses and also prepared a lawsuit.

2008: DOW AgroSciences petitioned US EPA to register CPF for additional agricultural uses.

2008: US EPA prepared a report for the FIFRA Scientific Advisory Panel (SAP) presenting the epidemiological evidence but left the then current safety standards intact. New science on infants, children, and pregnant women from experimental laboratory toxicology and epidemiology studies were examined.

2008: FIFRA SAP meeting to evaluate the Toxicology Profile for CPF.

2009-10: US EPA continued to gather epidemiological evidence data.

2010: Columbia researchers invited US EPA to a presentation of their 7 year findings from their CCCEH cohort (1998-2004).

2011: Preliminary human health risk assessment for registration review. In this document, US EPA stated that chlorpyrifos is not likely to be carcinogenic to humans based on the lack of evidence of carcinogenicity in studies in rats and mice and the absence of a mutagenicity concern. (US EPA, 2011a)

2011: Chlorpyrifos Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Modeling Linked to Cumulative and Aggregate Risk Evaluation System (CARES)

2011: US EPA does not further restrict CPF uses; US EPA Preliminary Human Health Risk Assessment released (US EPA, 2011a) The critical endpoints were BMDLs for 10% RBC AChEI in pups (PND 11 pups) or pregnant dams.

2012: Federal Peer Review on reports of the MRI and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological cohorts.

2012: FIFRA SAP Additional analysis on science on infants, children, and pregnant women from experimental laboratory toxicology and epidemiology studies.

2012: US EPA released a mitigation decision for CPF based on potential excess risks from spray-drift to bystanders.

2014: US EPA Revised Human Health Risk Assessment for registration review released (US EPA, 2014a). The critical endpoints are PBPK-PD-estimated human equivalent doses based on 10% RBC AChEI These human PoDs are similar to the PoD values based animal data in the 2006 and 2011 US EPA risk assessments. There is much objection from academic institutions, the public, and other groups for the continued use of AChEI as the basis for regulatory standards.

2015: DPR released draft risk characterization document for CPF for external scientific review.

2016: US EPA utilized the PBPK portion of the PBPK-PD model from the 2014 US EPA Revised Human Health Risk Assessment to predict CPF blood levels in women based on the expected exposure from crack and crevice during the period of the Columbia CCEH study. These predicted blood levels were compared with measured blood levels of CCCEH that resulted in ~2% lower Working Memory Index. . This is the first time that US EPA proposed CPF PoDs that were not for RBC AChE inhibition, but rather for predicting risk of neurodevelopmental outcomes. These PoDs were drastically lower (approximately 1000-6400-fold) than the PoD in the US EPA 2014 Revised Human Health Risk Assessment. The results were presented at the SAP April 2016 meeting (US EPA, 2016a; US EPA/SAP, 2016). The SAP supported US EPA on the use of the PBPK model as a tool for assessing internal dosimetry following exposure to CPF, but did not support the approach of using the Columbia CCCEH cohort cord blood data for deriving PoDs.

2016: US EPA followed the SAP recommendation to estimate the time-weighted average (TWA) concentrations of CPF in fetal blood based on presumptive CPF residential use on crack and crevice/hard surface at the time of the CCCEH study (1998-2004). Using forward dosimetry, the concentration of CPF in human blood was calculated from the PBPK model (Figure 1) assuming a total exposure of 2 hours per day for 30 days and a 10% decrease in blood levels of CPF per day. The model used the TWA blood estimates as internal dose to back calculate external doses as points of departure (PoDs) for infants, children, and adults. These PoDs for were approximately 150-9000-fold lower than the PoDs based on 10% RBC AChE inhibition in the earlier US EPA risk assessments (US EPA, 2016b).

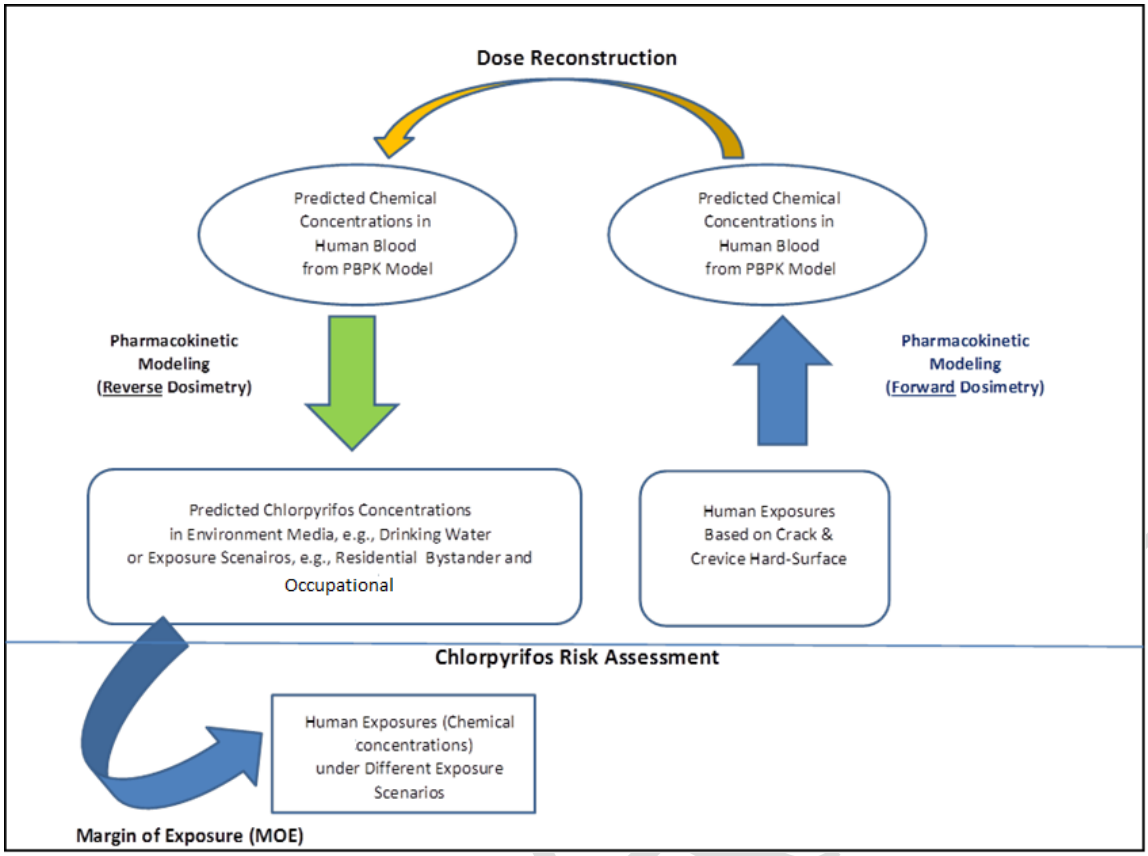


Figure 1. Depiction of the PBPK model incorporating estimation of CPF exposures

Based on Residential SOPs for crack and crevice and hard-surface exposures (www.epa.gov/sites/production/files/2015-08/documents/usepa-opp-hed_residential_sops_oct2012.pdf) from the same time-frame, predicted human blood CPF concentrations (dose reconstruction), and calculated exposures (reverse dosimetry) in the context of risk assessment (adapted and compiled utilizing the 2016 US EPA CPF PBPK model and exposure scenarios by Tan et al. (2007)

Scientific Advisory Panel

The FIFRA SAP convened several meetings to analyze the strengths and weaknesses of available data and to provide decision points on the incorporation of data for potential adverse neurodevelopmental effects in infants and children following prenatal CPF exposure. The first meeting in 2008 focused on a review of literature which reported associations of CPF exposure and adverse health outcomes in women and children (US EPA/SAP, 2008). Following this meeting, US EPA released a document detailing the aggregation of human data with other critical data and the determination of PoDs from human studies (Nolan et al., 1984; Rauh et al., 2006; US EPA/SAP, 2010; Rauh et al., 2011; Smith et al., 2011)

A proposal was made by Dow AgroSciences LLC to use a pharmacokinetic-pharmacodynamic model (PBPK-PD) developed for CPF PoD determination in risk assessment (Timchalk et al., 2002a; Timchalk et al., 2002b; Timchalk et al., 2005; Timchalk et al., 2006; Timchalk et al.,

2007; Timchalk and Poet, 2008). The SAP reviewed the model which was based on quantitative estimates of human AChE inhibition after oral, dermal, and inhalation exposure to CPF and CPF-oxon via dietary, water, occupational, and residential routes (US EPA/SAP, 2012). In its 2011 preliminary and 2014 revised CPF risk assessments, US EPA determined that AChE inhibition was the critical endpoint for CPF (US EPA, 2011a; US EPA, 2014a). This determination was based on the strength of the database as reflected by a statement by the SAP that:

> *"…AChE data provide the most appropriate endpoint and dose-response data for deriving PoDs for purposes of risk assessment. Moreover, because of the Agency's long experience with assessing the potential risk to CPF and other OPs, and because the dose response approaches based on AChE inhibition used in the 2011 preliminary assessment had been vetted by numerous SAPs, there was confidence in that approach*." (page 10)

Since 2012, the SAP has encouraged US EPA to evaluate both cholinergic (AChE) and non-cholinergic adverse endpoints, including developmental neurotoxicity and cognitive/behavioral alterations from CPF exposure (US EPA/SAP, 2012). Most notably, the revised 2014 US EPA risk assessment incorporated both a PBPK-PD model for deriving PoDs based on 10% RBC AChE inhibition, and evidence of neurodevelopmental effects in fetuses and children resulting from chlorpyrifos exposure as reported in epidemiological studies, particularly from the Columbia Center for Children's Environmental Health (CCCEH) cohort. At their April 2016 meeting, the SAP did not support using the cord blood data quantitatively for deriving PoDs. However, when considering the toxicological and epidemiological results, the panel concluded that there is evidence for adverse health outcomes associated with chlorpyrifos exposures below levels that result in 10% RBC AChE inhibition (US EPA/SAP, 2016).

California Proposition 65

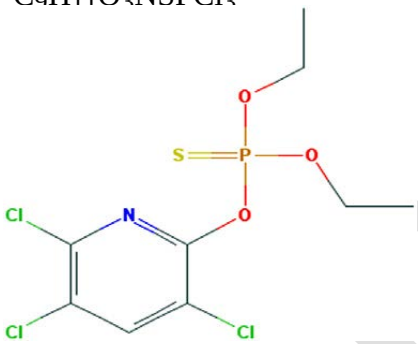
The Developmental and Reproductive Toxicant Identification Committee (DARTIC) agreed to consider whether chlorpyrifos should be listed under California Proposition 65 (the Safe Drinking Water and Toxic Enforcement Act of 1986) based on the developmental toxicity endpoint. At its meeting on November 29, 2017, the DARTIC agreed to list chlorpyrifos. Implementation is projected for 2018.

I.B.2. California Department of Pesticide Regulation (DPR)

CPF was given a "High" priority status by the California Department of Pesticide Regulation (DPR) due to concerns regarding 1) potential neurodevelopmental/ neurobehavioral effects from exposures during vulnerable developmental windows in fetuses, infants, and children, 2) genotoxicity and reproductive toxicity in rats, 3) probable human exposure due to spray drift, 4) possible infant exposure from hand-to-mouth activities, and 5) exposure through food and drinking water in California. Based on its "High" priority status, in 2011 CPF entered the DPR's process of comprehensive human health risk assessment (http://www.cdpr.ca.gov/docs/risk/raprocess.pdf and http://www.cdpr.ca.gov/docs/dept/prec/2011/prec_letter_report_52_20110916.pdf).

On July 1, 2015, CPF was designated as a restricted material when used as a pesticide product labeled for use in the production of an agricultural commodity.

I.C. Physical and Chemical Properties

Chemical Name:	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
CAS Number:	2921-88-2
Molecular Weight:	350.59 g/mol
Common Name:	Chlorpyrifos
Empirical Formula:	$C_9H_{11}O_3NSPCl_3$
Chemical Structure:	
Density:	1.51 ± 0.1 g cm^3 at 21 °C
Vapor Pressure:	2 x 10^{-5} mm Hg (0.003 Pa) at 25°C
Boiling Point:	> 320°C
Melting Point:	41–42°C
Flash Point:	> 200°F
Conversion Factor:	1 ppm = 14.31 ± 3 mg/m^3 at 25°C
Appearance:	Colorless to white, crystalline solid
Odor:	Mild mercaptan
Odor Threshold:	0.14 mg/m^3 (10 ppb)
Solubility in H_2O:	<2 mg/L solubility
Organic Solubility:	isooctane, methanol
Henry's Law Constant:	1 x 10^{-5} atm-m^3
Log Koc:	3.73
Kow:	4.8

I.D. Chemical Identification

CPF (Trade name- Dursban®, Lorsban®; O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate; CAS# 2921-88-2; DPR chemical code 253) is a crystalline broad-spectrum organophosphate (OP) insecticide that was first produced by Dow AgroSciences LLC in 1965. The toxic metabolite is CPF-oxon, generated by P450 activation and which inhibits acetylcholinesterase (AChE) in the nervous system (Meister and Sine, 2014; US EPA, 2014a).

I.E. Use and Product Formulations

I.E.1. Uses in California

Currently there are 48 actively registered product labels in California. Chlorpyrifos has been regulated in California as restricted use material since 2014

(http://www.cdpr.ca.gov/docs/legbills/rulepkgs/14-002/final_text.pdf and Table 1). By law, DPR requires the growers and pesticide applicators to report their pesticide use every year through their County Agricultural Commissioner. This pesticide use information can be found in the DPR Pesticide Use Reporting (PUR) database available at http://www.cdpr.ca.gov/docs/pur/purmain.htm. According to the most recent published data, total yearly use ranged from a low of 1.10 million pounds in 2012 to a high of 1.46 million pounds in 2013. The amount was applied over 0.9 – 1.3 million acres, with an average of 1 lb/acre, approximately the median application rate based on the label. There were no obvious trends in yearly use or acres treated. According to crop treatment data, the highest amount (in lbs) was compared to other crops (range: 192,482 in 2012 to 450,403 lb in 2013).

Table 1. Pesticide Use Data for CPF in California from 2011-2015

Year	Total yearly use (lb)	Total yearly treated (acre)	Top 5 crops treated	Yearly use for top 5 crops (lb)	Year	Total yearly use (lb)	Total yearly treated (acre)	Top 5 crops treated	Yearly use for top 5 crops (lb)
2011	1,296,074	1,186,979	Almond	231,067	2014	1,312,361	7,995,337	Almond	302,066
			Orange	205,595				Alfalfa	278,316
			Cotton	194,173				Walnut	187,152
			Alfalfa	185,879				Orange	162,986
			Walnut	163,097				Cotton	95,401
2012	1,100,873	1,051,292	Almond	192,482	2015	1,106,608	4,225,673	Almond	308,957
			Walnut	174,931				Orange	145,390
			Alfalfa	174,669				Walnut	133,242
			Orange	129,546				Alfalfa	123,748
			Cotton	97,769				Cotton	85,773
2013	1,465,115	9,889,464	Almond	450,403					
			Alfalfa	198,179					
			Walnut	166,340					
			Cotton	158,134					
			Orange	152,976					

I.E.2. Technical and Product Formulations

CPF is an AI in many registered products in various formulations, including emulsifiable concentrate, aqueous concentrate, flowable concentrate, ready-to-use liquid, wettable powder, pressurized liquid/fogger, paint/coatings, granular, microencapsulated, bait, and ear tag.

I.F. Human Illness and Exposure Reports

I.F.1. Reports of Human Illness

The California Pesticide Illness Surveillance Program (PISP) maintains a database of pesticide-related cases. An associated case is a record of one pesticide exposure and its apparent effects evaluated as definitely, probably, or possibly related to an exposure. A definite relationship indicates that both physical and medical evidence documents the exposure and consequent health effects. A probable relationship indicates that limited or circumstantial evidence supports a relationship to pesticide exposure. A possible relationship indicates that health effects correspond

generally to the reported exposure, but evidence is not available to support a stronger relationship. A case refers to a record of a pesticide exposure. An episode is an incident in which one or more people are exposed to the same source.

PISP receives reports of pesticide exposure from the California Pesticide Control System (CPCS), California Worker's Compensation, and from healthcare providers. PISP staff screen these reports and send the ones that meet program criteria to the County Agricultural Commissioners (CACs) for investigation. The CACs investigate the reports to determine if any violations of pesticide laws and regulations have occurred and collect information on the circumstances of exposure. The CACs send their reports to PISP for evaluation. PISP defines "agricultural" as pesticide use intended to contribute to production of an agricultural commodity including livestock. All other uses are considered "non-agricultural". PISP defines "occupational" as an individual who was on the job at the time of the incident and "non-occupational" as an individual who was not on the job at the time of the incident.

From 2004-2014, there were 246 associated cases of pesticide exposure stemming from 84 episodes involving chlorpyrifos. The number of illnesses varied throughout the 11 year period due to several multi-person episodes. Overall, the average number of chlorpyrifos episodes per year was 2.9. The average number of cases was 22.3 per year (Figure 2). The majority of illnesses were due to drift of pesticides (n=163, 66.2%), followed by residue (n=42, 17%). Ingestion accounted for 12 (5%) cases, eight of which resulted from improperly stored pesticides and/or those that were easily accessible to children (CDPR, 2017).

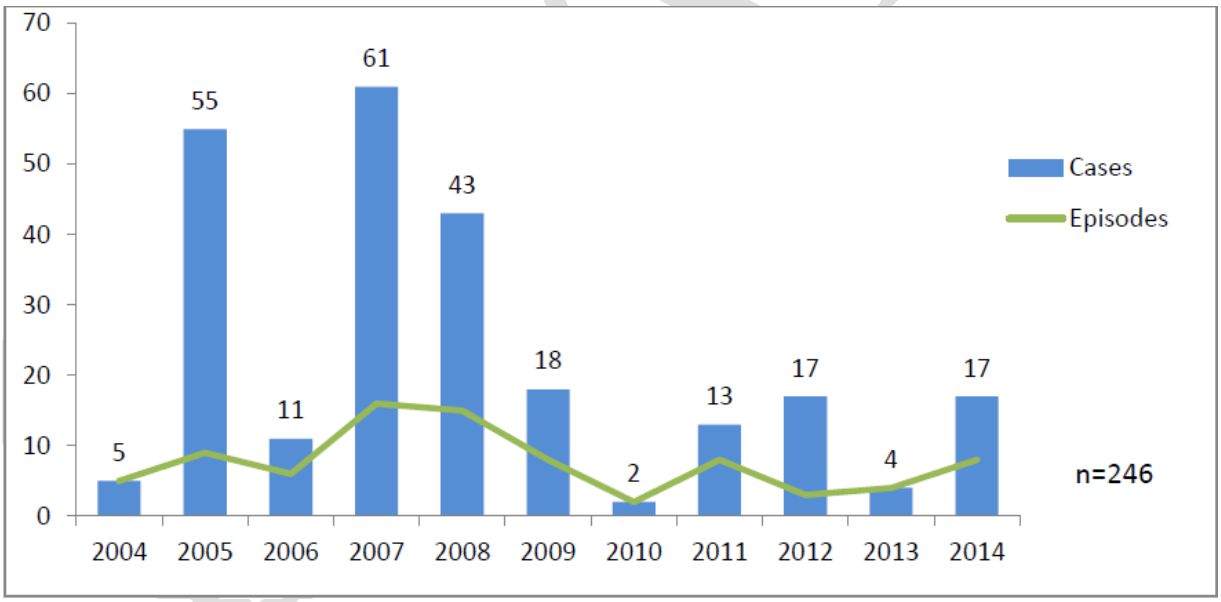


Figure 2. Cases and Episodes of Illness Due to Chlorpyrifos Exposure, 2004-2014

Bystanders accounted for 217 (88.6%) of the reported illnesses. Most bystanders were engaged in routine activities at the time of exposure (n=101, 41%), which meant they had minimal expectations of pesticide exposure. Fieldworkers followed with 82 cases (38%). Eighty-seven (35.6%) drift-related cases involved airblast sprayers, with the notable exception of 24 cases that involved chlorpyrifos used in combination with bensulfide applied by ground boom. Of the 246

cases involving chlorpyrifos in the years examined, 205 (83%) were agricultural and 40 (16%) were non-agricultural. Agricultural status could not be determined in one case. The majority of illness and injuries occurred while at work (n=171, 70%). Approximately, 60% (n=148) of the cases were both agricultural and occupational (Figure 3). Thirty-four cases involved children under the age of 18 (14%), 24 of which involved the agricultural use of chlorpyrifos (CDPR, 2017).

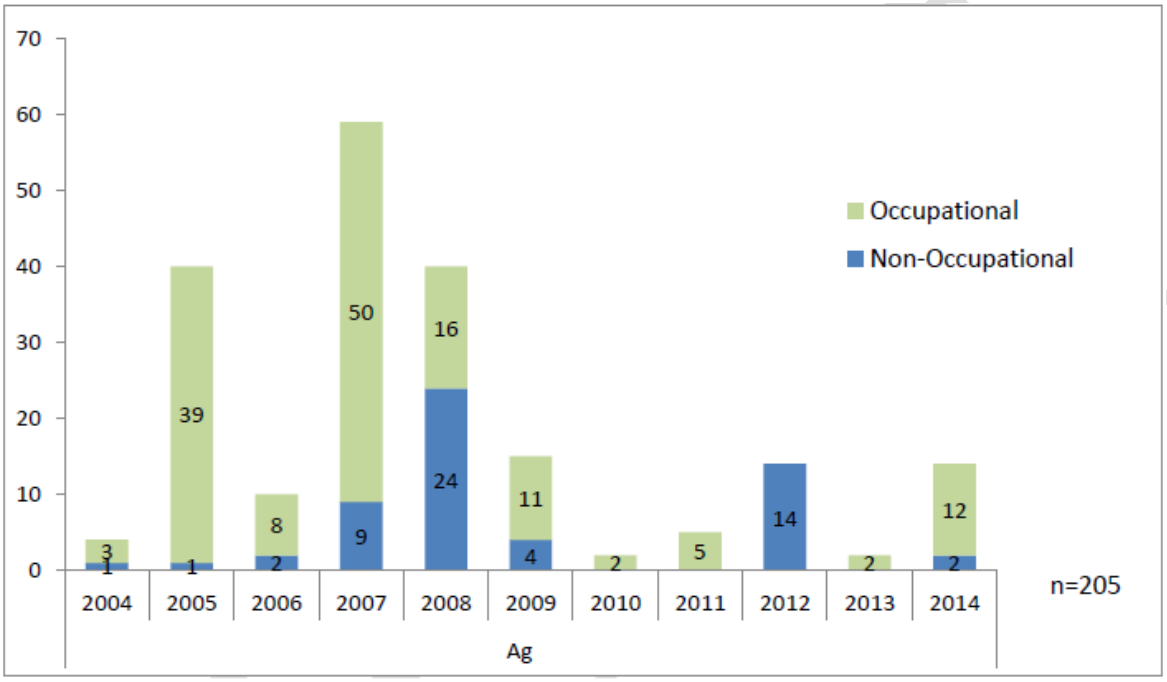


Figure 3. Chlorpyrifos Illnesses Caused by Agricultural Use, 2004-2014

Odor was also examined as a causal factor for reported symptoms. In agricultural drift episodes, the presence of an odor was the most frequently recorded contributing factor leading to illness, (n=147, 79%). Chlorpyrifos has a "skunky", rotten egg, garlic odor. Pesticides containing chlorpyrifos are often formulated with high percentages of petroleum based solvents, which can add to the odor. These solvents have a kerosene or gasoline-like smell. Unfortunately, most of the investigation reports did not provide a description of the odor in a way that would enable the distinction between the odor associated with chlorpyrifos and that of a petroleum-based solvent. The presence of an odor remains a significant concern, as it is suspected to potentially play a role in causation of symptoms experienced by people exposed to chlorpyrifos. Symptoms of exposure to these odorants include irritation to the eyes, nose and throat, dizziness, nausea, and headache. As such, it remains important to learn whether the odor from the petroleum distillates may be the source for symptoms experienced. DPR's Worker Health & Safety Branch recommends further investigation into the effect of the petroleum-based ingredients to help determine if some of these illnesses can be attributed to odor from the solvents. A summary the reported illness as well as episodes affecting five (5) or more people can be found in CDPR (2017).

I.F.2. Analysis of Human Exposure

Under the California Environmental Contaminant Biomonitoring Program (CECBP; http://biomonitoring.ca.gov), community studies are conducted in particular geographic areas or subpopulations that may be experiencing a common health outcome. Small pilot projects are designed to collaborate with laboratories and researchers on the collection and testing of urine and blood specimens from California residents. Through the program, four such biomonitoring studies were conducted to assess exposures to CPF in the environment by testing urine for 3,5,6-trichloro-2-pyridinol (TCPy), a urinary metabolite and exposure surrogate of CPF and CPM-methyl. While the results can be used to estimate the levels and probabilities of exposure in the represented populations, it is beyond the scope of these studies to associate levels of TCPy in urine with any specific health outcome. The studies are summarized below.

The Biomonitoring Exposures Study (BEST) Pilot study was jointly conducted by CECBP and the Kaiser Permanente Northern California (KPNC) Division of Research and part of a more extensive Kaiser Permanente Research Program on Genes, Environment, and Health (Das and Van Den Eeden, 2011). Urine and blood specimens were collected from 112 subjects from California's Central Valley in 2011 and 2012 for bioanalysis of analytes that included brominated flame retardants, environmental phenols, heavy metals, and pesticides, including the urinary metabolite TCPy. TCPy levels in urine that exceeded the limit of detection (LOD; 0.500 µg/L) were detected in 81% of 109 total specimens. The geometric mean of urinary TCPy was 1.23 µg/L. The BEST study was expanded to include 341 male and female adults from the Central Valley with expanded emphasis on Hispanic subjects and those from Asian/Pacific Island descent (DiBartolomeis, 2013). Urine and blood specimens were collected in 2013, although the data were not reported at the time of this publication.

The Firefighter Occupational Exposures (FOX) Project was jointly conducted by CECBP, the University of California (UC) Irvine Center for Occupational Health, and the Orange County Fire Authority (OCFA) (Das, 2010). The study was designed to quantify approximately 40 environmental chemicals in the blood and urine of Orange County, CA firefighters. A subset of chemicals was also analyzed in dust samples collected from three Orange County fire stations. Urine and blood specimens were collected from 101 subjects in 2010 and 2011. The environmental chemicals of interest included brominated fire retardants, perfluorinated chemicals, polychlorinated biphenyls, organochlorine pesticides, heavy metals, pesticide metabolites (including TCPy), and a polycyclic aromatic hydrocarbon metabolite. TCPy levels in urine that exceeded the LOD (0.500 µg/L) were detected in 89% of 101 total specimens. The geometric mean of TCPy detected was 1.78 µg/L.

The Maternal and Infant Environmental Exposure Project (MIEEP)-Chemicals in Our Bodies Project was jointly conducted by the UC San Francisco (UCSF) Program on Reproductive Health and the Environment, CECBP, and the UC Berkeley School of Public Health (Woodruff, 2009). The aims of the project were to assess exposures to environmental chemicals in 65 mother infant pairs and 27 pregnant women. English and Spanish speaking subjects were recruited at San Francisco General Hospital in 2010 and 2011. Urine specimens were collected in the third trimester of pregnancy while maternal and cord blood specimens were collected at parturition for bioanalysis. Environmental chemicals of interest included multiple compounds and metals, as well as pesticides and their metabolites (including TCPy). TCPy levels in urine specimens

exceeded the LOD (0.200 µg/L) and had a geometric mean of 0.52 µg/L with a 95% confidence interval bounded by 0.41 and 0.65 µg/L (N = 89).

Although several human epidemiological studies have also measured urinary TCPy and other general OP pesticide metabolites (Berkowitz et al., 2003; Eskenazi et al., 2004; Whyatt et al., 2009; Bouchard et al., 2011), there is no one background standard concentration that is currently used for comparison at this time and there is no reference concentration of urinary TCPy that this linked with a defined adverse health outcome.

The National Health and Nutrition Examination Survey (NHANES) is conducted by the Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/nchs/nhanes/biospecimens/serum_plasma_urine.htm). Some NHANES subset studies have analyzed for TCPy, including the NHANES-III subset of 1000 adults who were tested from 1988 – 1994. TCPy was detected in over 80% of the samples, with a median level of 2.2 µg/g creatinine (Hill et al., 1995). A subset of 80 adults were selected from the National Human Exposure Assessment Survey (NHEXAS-MD) and serially sampled in Maryland. TCPy was detected in 96% of samples with a median concentration of 4.6 µg/g creatinine (MacIntosh et al., 1999). In the Minnesota Children's Pesticide Exposure Study (MNCEPS), a Phase III special study that was part of NHEXAS, 102 children 3-13 years old were monitored for commonly used pesticides in 1997 (Adgate et al., 2001). TCPy was present in 93% of the samples and the mean urinary level was 9.2 µg/L. TCPy levels were significantly higher in urban than in nonurban children (7.2 vs. 4.7 µg/L, $p = 0.036$), although the sampling occurred before the US EPA ban on indoor application of chlorpyrifos.

Table 2. Summary of TCPy Levels Measured in Humans

Study	No. of subjects	% Samples with analyte detected	Urinary TCPy level*	Urinary TCPy concentration per g creatinine	Reference
BEST	112	81%	1.23 µg/L (GM)		Das and Van Den Eeden (2011)
FOX	101	89%	1.78 µg/L (GM)		Das (2010)
MIEEP	92	NA	0.52 µg/L (GM)		Woodruff (2009)
NHANES-III	1000	80%		2.2 µg/g	Hill et al. (1995)
NHEXAS-MD	80	96%		4.6 µg/g	MacIntosh et al. (1999)
MNCEPS	102 (children)	93%	9.2 µg/L		Adgate et al. (2001)

* GM, Geometric mean noted if available
NA = data not available

I.G. Environmental Fate

A review of the CPF environmental fate is presented in Koshlukova and Reed (2014) and is briefly summarized here. The half-life for interaction with photochemically generated hydroxyl radicals in air to produce dechlorinated products is 6.3 hours. CPF is spontaneously degraded by photolysis and hydrolysis in soil and water and can persist from 2 weeks to 1 year, depending on soil type, climate, and presence of soil microbes. Hydrolysis products including TCPy and

phosphorthioic acid may form under alkaline conditions. Hydrolysis is increased with increased temperature and alkalinity of the water source (e.g., river or water well; $T_{½}$ = 4.8 to 38 days). The Log K_{oc} (3.73) indicates that CPF adsorbs strongly in soil and resists leaching to ground water. CPF will persist for weeks or months in indoor environments (Berkowitz et al., 2003; Rauh et al., 2006; US EPA, 2014a). In the environment, CPF is oxidized to the toxic metabolite CPF-oxon by photolysis, aerobic metabolism, and chlorination (e.g., drinking water). The CPF K_{ow} (4.8) indicates a potential for bioaccumulation in aquatic (TCPy and conjugates detected in fish tissues) and terrestrial food chains. Information on chlorpyrifos environmental fate from the DPR Environmental Monitoring branch can be found here: http://www.cdpr.ca.gov/docs/emon/airinit/2560_chlorpyrifos_final.pdf and http://www.cdpr.ca.gov/docs/emon/pubs/tac/tacpdfs/chlrpfs/append_a_chlorpyrifos_use_information.pdf

II. TOXICOLOGY PROFILE

Chlorpyrifos (CPF) is a chlorinated organophosphorus (OP) ester used as an insecticide, acaricide, and miticide. The toxicity of CPF is associated with binding and inhibition of the enzyme acetylcholinesterase (AChE) in insects and mammals. CPF requires metabolic activation to CPF-oxon to yield anticholinesterase activity. CPF causes developmental neurotoxicity at exposure levels that do not induce overt toxicity or inhibit cholinesterase (ChE) activity. CPF has major uses in California as an insecticide for nut trees, fruit, vegetable, and grain crops as well as non-food crop scenarios (e.g., golf course turf, industrial sites, greenhouse and nursery production, sod farms, and wood products).

An overview of the toxicity of CPF is presented below. The studies evaluated were submitted by the registrant and/or obtained from the open literature. More detail of the registrant-submitted studies and other studies contributing to the hazard assessment can be found in the HHA Summary of Toxicology Data (Appendix 1) and in the US EPA 2011 Preliminary Human Health Risk Assessment for Reregistration and in the US EPA 2014 Revised Human Health Risk Assessment (US EPA, 2011a; US EPA, 2014a).

II.A. Acetylcholinesterase Inhibition

AChE normally breaks down the neurotransmitter acetylcholine (ACh) within the central nervous system (CNS) synaptic cleft or at neuromuscular or neuro-glandular junctions in the peripheral nervous system (PNS) (Casida and Quistad, 2004; Testai et al., 2010). The active metabolite of CPF is CPF-oxon, which inhibits AChE by binding at the active site. When AChE inhibition occurs, ACh accumulates and results in unremitting nerve impulses that lead to continuous muscle responses in the peripheral nervous system (PNS) or neural stimulation in the central nervous system (CNS).

Cholinesterase exists in plasma in the form of BuChE. However, in red blood cells cholinesterase only occurs as AChE and in the brain primarily as AChE (Eaton et al., 2008; Testai et al., 2010). In the rat brain, AChE activity is higher than BuChE activity (90% versus 10% of total)(Mortensen et al., 1998; Li et al., 2000b). The BuChE:AChE ratio varies with species, with a ratio of 1000:1 in humans, 7:1 in dogs, 2:1 in female rats, and 1:3 in male rats (Scarsella et al., 1979; Brimijoin, 1992).

In general, HHA considers brain cholinesterase inhibition to be indicative of overt toxicity not only because the brain is a primary functional target site, but also because more subtle central neurological signs such as memory and learning losses may not be easily detected or quantified. In contrast, the toxicological significance of AChE inhibition in plasma and RBCs is less certain because the physiological function of cholinesterase in blood has not been clearly established. Plasma cholinesterase, or more specifically BuChE, may be involved in the binding or metabolism of certain drugs, suggesting that BuChE inhibition may compromise an organism's ability to defend against subsequent toxic insults (Lockridge and Masson, 2000). BuChE is also the predominant form of cholinesterase in the developing nervous system of birds and mammals (Brimijoin, 1992). Other evidence suggests that BuChE may also play a role in the co-regulation of ACh levels in the adult nervous system (Li et al., 2000a). Gene-targeted mice deficient in AChE ($AChE^{-/-}$) showed that BuChE and likely other enzymes may have assumed the function of AChE during early development(Li et al., 2000a; Xie et al., 2000). The $AChE^{-/-}$ mice showed no physical defects at birth. Their organs and blood cells showed no morphological abnormalities. Electron microscopic examination of the neuromuscular junctions showed normal morphology. Interestingly, BuChE levels in the tissues were similar to those in the wild-type and AChE heterozygous mice. In addition, in the absence of AChE, BuChE was apparently essential for vital functions. When $AChE^{-/-}$ mice were treated with bambuterol, a specific BuChE inhibitor, they died immediately after treatment, while wild-type mice treated with the same dose were not affected. Despite the low levels of BuChE in the brain, BuChE in the brain of $AChE^{-/-}$ mice may help maintain a minimal level of cholinergic function by hydrolyzing extrasynaptic acetylcholine.

Although blood cholinesterase inhibition is generally not considered detrimental, it may be a useful surrogate for brain and/or peripheral AChE inhibition (US EPA, 2000a). This is because blood cholinesterase inhibition occurs well before brain AChE inhibition. Therefore, protecting inhibition in blood may potentially protect the downstream effects in the brain and peripheral nervous system (Nolan et al., 1984). RBC AChE inhibition data are generally preferred over BuChE inhibition data because RBCs contain only AChE whereas plasma can contain both BuChE and AChE (Testai et al., 2010). This is important in determination of no-observed-effect-levels (NOELs) or PoDs because CPF may have considerably different affinity for the active site of BuChE versus AChE (US EPA, 2000a).

The Joint Meeting on Pesticide Residues of the World Health Organization (WHO) concluded that RBC AChE inhibition should only be used as a surrogate for peripheral cholinesterase inhibition at the time of peak effect with acute exposure since RBCs lack the ability to synthesize new AChE (Brimijoin, 1992; WHO/JMPR, 1999). Consequently, the recovery of RBC AChE activity is much slower than in neurological and neuromuscular tissue because it is dependent on the replacement of RBCs. HHA is currently reevaluating the use of cholinesterase inhibition data in its risk assessments. In anticipation of changes in the use of these endpoints, NOELs for blood and brain inhibition were identified in this document based on statistical significance.

II.B. Metabolism and Pharmacokinetics

Numerous articles have described the metabolism of CPF in animals and humans (Timchalk et al., 2002a; Timchalk et al., 2002b; Timchalk et al., 2005; Timchalk et al., 2006; Eaton et al.,

2008; Timchalk and Poet, 2008; Testai et al., 2010). P450s oxidize CPF to form an unstable phosphooxythiiran intermediate that undergoes oxidative desulfuration to form CPF-oxon. Additionally, dearylation (oxidative ester cleavage) of the intermediate results in the formation of TCPy and diethylthiophosphate (DETP) (Figure 4). The active metabolite CPF-oxon can inhibit AChE or form TCPy, the latter of which is considered the detoxification pathway. The balance of CPF activation to detoxification is dependent on species, gender, age, P450 enzyme profiles, and P450 enzyme polymorphisms (Ma and Chambers, 1994).
CPF-oxon is formed in humans when CPF is metabolized by three main forms of P450:

Activation of CPF→ CPF-oxon by CYP2B6 (desulfuration)
Activation of CPF→ CPF-oxon by CYP3A4/5
Detoxification of CPF → TCPy by CYP2C19 (dearylation) and CYP3A4/5

CPF-oxon is unstable and can be further metabolized by calcium-activated A-esterases (PON1) and B-esterases (BuChE and carboxyesterases) in blood, brain, liver, and other tissues (Figure 4) (Testai et al., 2010). These enzymes can detoxify CPF-oxon before it inhibits AChE in the central or peripheral nervous systems. The A and B-esterases as well as P450s can detoxify CPF-oxon to form the urinary metabolite 3,5,6-trichloro-2-pyridinol (TCPy) which has served as a biomarker for CPF metabolism (Testai et al., 2010). TCPy is a product of both the activation and detoxification pathways and therefore cannot be directly associated with toxicity.

Detoxification of CPF-oxon → TCPy by PON1 and ChE

TCPy in urine can also indicate exposures to CPF-oxon, CPF-methyl and triclopyr (Barr and Angerer, 2006; Whyatt et al., 2009). Environmental, dietary and home exposure to TCPy can occur as a degradate of CPF, CPF-oxon or CPF-methyl (Barr and Angerer, 2006; Eaton et al., 2008; Whyatt et al., 2009). Significant intra-individual variability in repeat urine samples from the same individual has been observed (Whyatt et al., 2009).

PON1 activity is generally less in newborns than in adults. PON1 activity increases approximately 3.5 fold until age 7, when activity levels are closer to those found in adults (Cole et al., 2003; Holland et al., 2006; Huen et al., 2010). PON1 polymorphisms [glycine (Gln; *Q* allele) to arginine (Arg; *R* allele) substitution] have esterase activities that are substrate-dependent(Ginsberg et al., 2009). These alleles and phenotypes develop at different ages, and these developmental differences affect the age-dependent pharmacokinetic disposition and age-dependent pharmacodynamic activities of CPF (Huen et al., 2010).

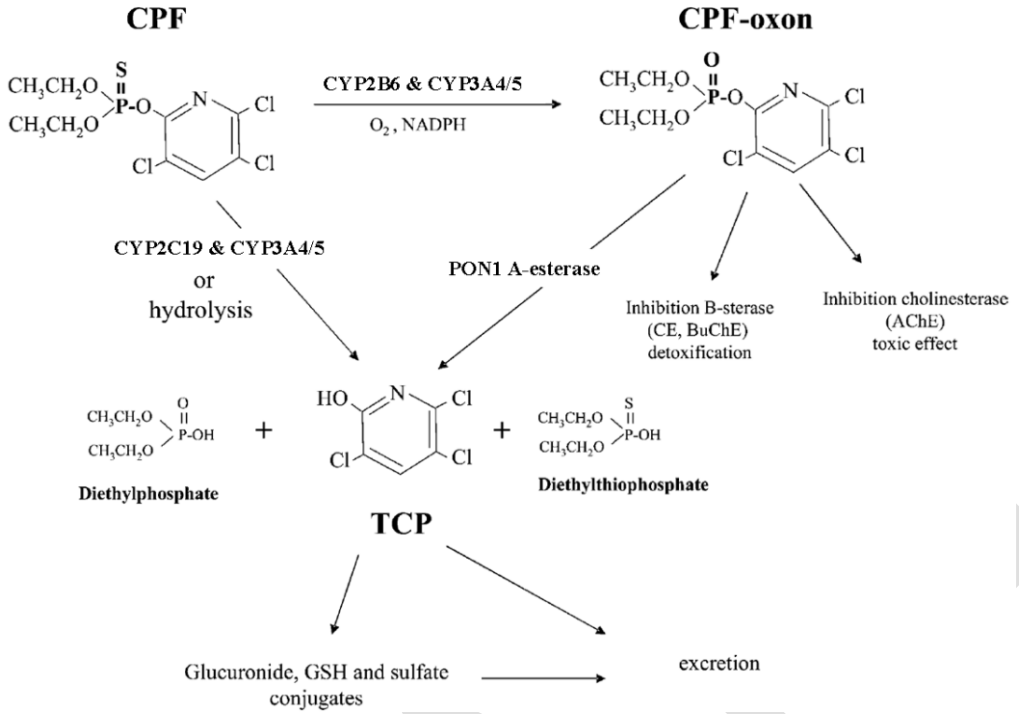


Figure 4. The Major Metabolic Pathways for CPF
(Adapted from Testai *et al.*, 2010)

II.B.1. Metabolism and Pharmacokinetics in Rat

Nolan et al. (1987): ^{14}C-labeled CPF was administered via gavage to Fischer 344 rats (5/sex/dose) in corn oil (2 ml/kg) in a single labeled dose of 0.5 or 25 mg/kg or via 15 consecutive daily doses of unlabeled CPF at 0.5 mg/kg/d followed by a single 0.5 mg/kg dose of ^{14}C-labeled CPF. The ^{14}C label was on the TCPy moiety. Investigators evaluated ^{14}C levels in urine, feces, and tissues and identified the three significant urinary metabolites. Urine plus cage wash accounted for 86 – 93% of administered dose regardless of sex or dosing regimen (~100% absorption). Six to 11% of the total administered ^{14}C was detected in feces. Urinary excretion was rapid, with over 50% of the administered dose collected in urine usually within the first 12 hours. $T_{1/2}$ was 8 – 9 hours for single or multiple 0.5 mg/kg treatment groups and somewhat longer for the 25 mg/kg group. Urinary metabolites were comprised chiefly of TCPy. Together with the glucuronide conjugate, TCPy accounted for over 90% of urinary metabolites. About 5% of urinary residues consisted of the sulfate conjugate of TCPy. Parent CPF was not found in urine. Most fecal ^{14}C was obtained within the first 24 hours. Exhaled CO_2 from the 25 mg/kg group was trapped for radioanalysis and accounted for < 0.01% of administered dose. Fecal metabolites were not assessed. Tissue residues were assessed at 72 hrs for males and 144 hrs for females. Total tissue residues were small to negligible, accounting for only 0.2% of administered dose in 25 mg/kg group and < 0.01% in all other groups. These residues were generally only quantifiable in peri-renal fat in both sexes.

Marty and Andrus (2010): Rat pups (post-natal day [PND] 11) and young adult female Sprague-Dawley rats (70-80 days old) were dosed with CPF or CPF-oxon as an acute (single) or

repeat dose (11 days). **CPF Treatment:** Acute gavage CPF dose regimen in pups (8/sex/dose/group) was 0, 0.05, 0.1, 0.5, 2 and 5 mg/kg (in corn oil vehicle [c.o.] or rat milk) and adults it was 0 (corn oil vehicle or in diet; 8/dose/group), 0.05, 0.1, 0.5 or 10 mg/kg. Repeat gavage CPF dosing in pups (8/sex/dose) and adults (8/dose) was 0 (c.o.), 0.05, 0.1, 0.5, 1, or 3.5 mg/kg/d. **CPF-oxon Treatment**: Acute gavage CPF-oxon dose regimen in pups was 0 (c.o.), 0.005, 0.01, 0.05, 0, or 0.5 mg/kg and in adults it was 0 (c.o.), 0.05, 0.1, 0.5, or 10 mg/kg. Repeat gavage dosing in pups and adults was 0 (c.o.), 0.01, 0.5, 1, or 3.5 mg/kg/d. **Methods:** Preliminary studies were performed in order to establish the time-to-peak inhibition profile for plasma, red blood cell, and brain cholinesterase inhibition. In the dose-response studies, animals were euthanized at the time-to-peak cholinesterase inhibition. The concentrations of CPF, CPF-oxon, and TCPy in the blood of selected animals were determined. A functional observational battery was performed on the study animals in the multiple-dosing regimen after 9 days of dosing. **Results:** Untreated pups showed no significant differences among plasma, RBC, or brain cholinesterase activity and there were no differences in the enzymes activities between males and females. In pups, plasma cholinesterase was 4.5 times less active than RBC AChE, while brain AChE activity was 3.7 times higher than RBC AChE activity. For adults, RBC AChE was 2.6 more active than in plasma, but brain AChE activity was 9.6 times higher than RBC AChE. Both plasma cholinesterase and brain AChE were higher in adults than in pups, however RBC AChE activity was lower in adults than pups. The measured time-to-peak enzyme effects were as follows:

Animals	Dose	Time to peak enzyme effect
Rat pups	CPF in corn oil vehicle	6 hrs
	CPF-oxon in corn oil vehicle	4 hrs
	CPF in rat milk vehicle	8 hrs
Adult rats	CPF in corn oil vehicle	8 hrs
	CPF-oxon in corn oil vehicle	4 hrs
	CPF in diet (after 12-hr exposure period)	8 hrs

Based upon the results of the dose response studies, no effect levels were established for plasma, RBC, or brain AChE inhibition under the different dosing scenarios. In the single dose regimen, NOELs for plasma and RBC AChE inhibition were 0.5 mg/kg for both sexes of pups after treatment with CPF (in corn oil or rat milk vehicle) and in adults (in corn oil or in diet). The NOEL values for brain AChE inhibition were 2 mg/kg for the male pups treated with CPF (in corn oil or rat milk vehicle), as well as for the female pups and adults (corn oil vehicle only). For the pre-weanling females dosed with CPF in the rat milk vehicle, the brain AChE inhibition NOEL was 0.5 mg/kg. The NOELs from a single dose of CPF-oxon to pups were 0.05 mg/kg for plasma cholinesterase inhibition, 0.1 mg/kg for RBC AChE inhibition, and 0.5 mg/kg for brain AChE inhibition. For the adults, the NOEL for plasma, RBC, and brain AChE inhibition were 0.1, 0.1, and 0.5 mg/kg, respectively. In the multiple dose regimen in which the pups and adults were treated with CPF in corn oil by gavage, the NOEL values for cholinesterase inhibition in pups were 0.1 mg/kg in plasma and RBCs and 0.5 mg.kg in brain. For adults, the NOEL values were 0.1 mg/kg/d for plasma, 0.5 mg/kg/d for RBCs, and 0.5 mg/kg/d for brain. The NOELs for ChE inhibition in both pups and adults after multiple treatments with CPF-oxon in corn oil were 0.01 mg/kg/d in plasma and RBCs and 0.5 mg/kg/d in brain. The NOEL values were reduced from 0.5 mg/kg to 0.1 mg/kg/d for plasma and RBC ChE inhibition in the pre-weanlings after

multiple treatments with CPF in corn oil. The brain AChE inhibition for these animals was lowered from 2 mg/kg to 0.5 mg/kg/d. In the young adult females, the NOELs for plasma and brain ChE inhibition were lowered from 0.5 mg/kg to 0.1 mg/kg/d and from 2 mg/kg to 0.5 mg/kg/d, respectively. The concentrations of CPF and TCPy in the blood at the NOEL and/or LOEL treatment levels for the various treatment scenarios were examined. Treatment with CPF in corn or in rat mild to pre-weanling rats in either a single dose or multiple dose regimen resulted in TCPy/CPF concentration ratios ranging from 70 to 209 ng/g of blood. In certain instances, the CPF concentration in young female rats was below the LOD and the ratio could not be calculated. Otherwise, the ratios were 935 and 449 (0.5 and 2.0 mg/kg, by gavage, respectively), 7243 (2.0 mg/kg in the diet) in the single dose regimen, and 2450 (0.5 mg/kg/d) and 651 (1.0 mg/kg/d) after multiple doses by gavage. These data indicate a possible difference in the metabolic disposition of CPF between the pre-weanling pups and the young adult animals. No treatment-related effects were identified in the FOB. Study deficiencies include the limited sample sizes with which to analyze CPF (2 pups, 4 adults), CPF-oxon and TCPy in blood, which led to increased variability. Therefore it was difficult to find a correlation between blood levels of these compounds and AChE inhibition. Analyses were performed at peak effect levels. Because only CPF-oxon is the active inhibitor, correlation with blood levels of CPF and TCPy with inhibition is difficult to interpret.

Mattsson et al. (1998); Mattsson et al. (2000b): Pregnant Sprague-Dawley rats were gavaged at 0 (corn oil), 0.3, 1.0 or 5.0 mg/kg/d from gestation day (GD) 6 to postnatal day (PND) 10. On GD 20 (4 h post gavage), fetal CPF in blood (46 ng/g blood) was half that of dams (109 ng/g blood) at 5.0 mg/kg/d. CPF-oxon was detected only once in fetuses (1 ng/g blood). No blood CPF was detected in dams (limit of quantitation 0.7 ng/g); however, there was significant plasma and RBC AChE inhibition at 0.3 mg/kg/d. This is likely due to production of CPF-oxon metabolized from CPF in blood. In contrast, fetuses of dams at 1 mg/kg/d had a detected blood CPF (1.1 ng/g); without ChE inhibition in any tissue. Inhibition of AChE was greater in dams at all doses but occurred only at 5.0 mg/kg/d in fetuses. At 5.0 mg/kg/d the inhibition was RBC > plasma > heart > brain (least inhibited). At 5.0 mg/kg/d milk CPF was 200-fold greater than in blood and pups were exposed in milk at approximately 0.12 mg/kg/d. Nursing pup exposure was lower than that of dams and AChE inhibition at 5.0 mg/kg/d was back to control levels by PND 5. The authors of this article concluded that "Based on the lesser ChE inhibition in fetuses, and on estimates of CPF consumption in milk, neither fetuses nor neonates demonstrated greater sensitivity to ChE inhibition than their dams."

Hotchkiss et al. (2010) ***Phase I:*** Sprague-Dawley rats (6/sex/dose) were exposed to CPF via nose-only inhalation to 0, 13.3 or 66.7 mg/m^3 for six hours. Blood was drawn from an in-dwelling jugular catheter at 2, 4 and 6 hours of exposure and at 0.5, 1, 2, 4, 6, 12, and 24 hours post-exposure. Red blood cell and plasma ChE activities were assayed for each time point. ***Phase II:*** Female rats (54/dose) were exposed via nose-only inhalation to CPF at 0, 3.7, 12.9, 22.1 or 53.5 mg/m^3 for up to 6 hours. Rats (6/dose/time point) were euthanized at 2, 4, and 6 hours of exposure and at 2, 6, 12, 24, 48 and 72 hours post-exposure. ChE activities in RBCs, plasma, lungs and brain were assayed and the blood concentrations of CPF, CPF-oxon and TCPy were measured. Urine was collected (6/dose) at 0-12, 12-24, 24-48 and 48-72 hours and TCPy concentrations were determined. ***Results***: In Phase I, significant RBC and plasma ChE inhibition was evident at 13.3 mg/m^3. RBC AChE had a peak inhibition of 65% (males) and 80% (females) at 2 hours post-exposure. Plasma ChE had a peak inhibition of 66% (males) and 87%

(females) occurred at 6 hours of exposure to 1 hour post-exposure. Based on these results, females were deemed to be more sensitive to the effects of CPF on ChE activity and thus were selected for testing in Phase II. ***Phase II:*** Plasma ChE inhibition was at a maximum of 48% at 6 hours of exposure in the 3.7 mg/m^3 group. In the lungs, a maximal level of AChE inhibition was 47% at 3.7 mg/m^3 at 6 hours of exposure. Brain AChE was significantly inhibited at 12.9, 22.1 and 53.5 mg/m^3; with maximal inhibitions of 19, 21 and 22% at 6, 6 and 2 hours post-exposure, respectively. For RBC AChE activity, the results were inconsistent at 3.7 mg/m^3 possibly due to the variability of the control values. Maximal AChE inhibition was not evident until 24 to 48 hours post-exposure. CPF in blood was highest at 4-6 hours of exposure for at all doses (peak value 65 ng/g at 53.5 mg/m^3). CPF-oxon was recovered in the blood (peak: 0.22 ng/g) during exposure at 53.5 mg/m^3. Peak levels of 2400 ng/g of TCPy for the highest exposure occurred at 12 hours post-exposure. The plasma half-life ($t_{1/2}$) of CPF was 0.463-3.34 hours over the exposure concentration range. The ratio of the areas under the curve for TCPy/CPF ranged from 545 to 1057. The inhaled dose of the test material was calculated to be 1.04, 3.62, 6.21 and 15.0 mg/kg. Excretion of TCPy in the urine $t_{1/2}$ was 10.6-11.6 hours. Using these excretion data the percentage of inhaled CPF which was absorbed was approximately 36-79%. An inhalation NOEL was not achieved due to increased plasma ChE and RBC AChE at 3.7 mg/m^3 (LOEL ~1.0 mg/kg/d inhaled dose).

Hotchkiss et al. (2013): Crl:CD(SD) female rats (40/dose) were exposed via inhalation (nose-only) at 0 (filtered air) or 17.7 ppb (0.254 µg/l) of a saturated vapor of CPF for 6 hours. Females (8/dose/time point) were euthanized at 0, 2, 4, 6 and 12 hours post-exposure. Blood, brain and lung tissue were procured from each animal. ChE activity (plasma, RBC, brain and lungs), as well as CPF, CPF-oxon and TCPy (in blood), were assessed. Females had no signs of toxicity during the exposure or for 12-hour post-exposure. Peak CPF in blood occurred immediately after completion of exposure; diminishing to a non-detectable level by 6 hours post-exposure. TCPy peak occurred up to 2 hours post-exposure and gradually diminished over the next 12-hours post-exposure. CPF-oxon was not detectable in any of the samples; however it may have been totally degraded before assessment. None of the tissues which were assayed from the exposed group demonstrated a significant decrease in AChE activity compared to controls. Activity in the blood and plasma of the exposed animals was 93 and 86%, respectively, of the control values at 4 hours post-exposure, the maximal reduction. The ChE activity in the lungs of the exposed animals was 89% of the control group at that time point. There was no apparent effect upon AChE activity in the brain.

II.B.2. Metabolism and Pharmacokinetics in Humans

II.B.2.a. Human Oral Studies

Kisicki et al. (1999): ***Part 1:*** Six male and six female human volunteers/treatment group were fasted overnight prior to being dosed orally once with 0 (placebo: lactose monohydrate), 0.5 or 1.0 mg/kg of CPF powder (purity: 99.8%) in capsules (phase 1) or 0 or 2.0 mg/kg (phase 2) in a double blind, randomized study. The health status of each subject was monitored for up to 7 days. Vital signs (blood pressure, pulse rate, respiration rate, and body temperature) were recorded prior to dosing and at 1, 2, 4, 8, 12, 24, 48 and 168 hours after dosing. Blood samples for RBC AChE analysis were drawn 10 hours prior to dosing, at the time of dosing and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hours post-dose for RBC AChE activity and CPF and

metabolite analyses. A blood sample was drawn prior to dosing for PON1 activity determination. Urine samples were collected at 12 hour intervals starting 48 hours prior to dosing and at 0 to 6 and 6 to 12 hours post-dose and 12 hour intervals thereafter up to 168 hours after dosing. Although clinical symptoms such as anorexia, diarrhea, nausea, vomiting, dizziness, dyspnea, and headache were reported, none of these signs occurred in a dose-related manner. There was no apparent treatment-related effect upon any of the vital signs. Mean RBC AChE activities were not significantly affected in a dose-related manner. One subject in the 2.0 mg/kg treatment group demonstrated a maximal 30% inhibition between AChE activity reported at 0 and 12 hours post-dose. Otherwise, no other subject in the high dose group had a reduction in RBC AChE activity greater than 12% based on the higher of the two baseline values. The blood and urine levels of CPF and its metabolites and the paraoxonase activity analysis for individual subjects were not included in this initial report and thus could not be evaluated. No adverse effects were indicated. **NOEL:** 1.0 mg/kg (based upon the 30% inhibition of RBC AChE demonstrated by one of the subjects in the 2.0 mg/kg treatment group). ***Part 2:*** As a continuation of the above study, 30 days after the oral treatment, the human volunteers (6/sex/dose) received a single oral dose of 0.0, 0.5, 1.0 or 2.0 mg/kg (capsule form) in a double-blind clinical trial; blood and urine specimens were collected and analyzed for CPF and its metabolites (CPF-oxon and TCPy) using gas chromatography-mass spectrometry (GC-MS). CPF paraoxonase (PON1) prior to treatment was determined spectrophotometrically. The blood and urine specimens were generally below the limit of quantitation (LOQ) for CPF. An average area under the curve for TCPy in blood (by increasing dose) was 14.0, 25.2 and 51.2 µg/g, respectively. TCPy excreted in the urine was 4.1, 8.7 and 15.9 mg, by dose, respectively, during the first 168 hr following ingestion; Blood and urinary TCPy levels increased rapidly, remained constant over first 48 hr post-treatment, and then declined with an average half-life of 29 to 36 hours. Administration by capsule probably reduced absorption (average of 34.7%, 30.8% and 29.5% absorbed in 0.5, 1.0 or 2.0 mg/kg dose group, respectively). The serum CPF PON1 activity was within the range of activity reported in previous studies and there were no extreme values. RBC AChE inhibition was seen in only one individual (female at 2.0 mg/kg) that showed unusually high absorption of CPF (87.9% versus 29.5%).

II.B.2.b. Human Oral Treatment and Dermal Absorption Studies

Nolan et al. (1982); Nolan et al. (1984): Researchers selected healthy male volunteers (n = 5) to characterize CPF kinetics and production of the major metabolite TCPy, and to follow changes in plasma and RBC AChE over time. Exposures were a 0.5 mg/kg single oral dose, followed 4 weeks later by a single 5 mg/kg dermal dose. None of these doses elicited clinical signs. Following 0.5 mg/kg oral dosing, plasma ChE was inhibited to about 15% of baseline, with the greatest inhibition at 0.5 to 2 hrs after dosing. By 8 hours, plasma ChE activity levels were 3-4-fold higher than the lowest activity. By 27-30 hours, plasma ChE activity returned to baseline activity. Dermal dosing with 5 mg/kg CPF had no definitive effect on plasma ChE at any time post-dose. RBC AChE activity was not measurably affected by these oral or dermal exposure levels. Blood CPF levels following 0.5 mg/kg oral dosing was either non-detectable, or was in the range of 5-30 ng/ml blood. The highest blood CPF levels did not appear at consistent times post-dosing, and clearly would not represent a reliable measure of exposure. Blood concentrations of CPF following 5 mg/kg dermal exposure were either non-detectable or did not exceed 10 ng/ml. Blood levels of TCPy following 0.5 mg/kg oral dosing showed quite variable kinetics between subjects, but tended to peak at 2-8 hours at about 1 µg/ml blood, with levels at

24 hours being no less than 50% of peak concentrations. This confirms that this metabolite would be a reliable indicator of exposure. Dermal exposure of 5 mg/kg yielded TCPy blood levels which occasionally exceeded 0.1µg/ml. There was about a 4-fold range of peak TCPy blood between dermal exposure subjects. Investigators estimated the half-life of TCPy to be about 27 hours by either route. Urinary peak excretion rates of TCPy were at about 9 hours for oral route, and about 42 hours for the dermal route. Time to decrease to about 50% of maximum urinary TCPy levels were roughly 30 hours for oral exposure and 84 hours for dermal route. This study showed that CPF is only moderately absorbed through the skin (1.28% absorption), that plasma ChE is a good marker of systemic load for several hours after exposure, whereas urinary TCPy assays would be useful for qualitative exposure assessment for 2-3 days for oral route and slightly longer for dermal exposure.

Griffin et al. (1999): A human volunteer study (n = 5; 4 men, 1 woman) was performed with CPF to determine the kinetics of urinary excretion of dialkylphosphate (DAP) metabolites and plasma and RBC AChE inhibition after oral (1 mg) treatment, followed one month later with dermal (28.59 mg; 8 hrs) treatment. After 8 hours skin was washed and the CPF residue was collected for analysis. After both oral and dermal treatments blood was collected over 24 hours. Plasma and RBC AChE concentrations were determined for each sample. Urine was collected for 100 hours and the CPF metabolites (DAPs) were assayed in each urine sample. Elimination half-life for DAPs in urine after oral dosing was 15.5 hours and 30 hours for dermal dosing. Average recoveries were 93% and 1% for oral and dermal dosing, respectively. Dermal dose recovery from the skin surface was 53% and 456 ng/cm^2/h based on urinary DAPs. ChE (plasma or RBC) was not significantly inhibited after oral or dermal exposure. CPF exposure was indicated only through urinary DAPs in this study.

II.B.2.c. Human Dermal Absorption Studies

Meuling et al. (2005): Dermal absorption of CPF in humans was assessed by urinary elimination of TCPy. Male volunteers were administered CPF dermally (100 cm^2) at 5 mg or 15 mg (n = 3/dose) for 4 hours. Subsequently, the unabsorbed CPF residue was washed off. At designated intervals, CPF and TCPy were assessed in the dosing and wash solutions and in urine samples up to 120 hours post-dosing. Most of the treatment dose was found in "wash-off" from the skin (42%–67%). At 5 mg and 15 mg CPF, the urinary TCPy was 131.8 µg and 115.6 µg, respectively at 120 hrs post-dosing. Approximately 4.3% of the applied dose was absorbed as indicated by the lack of significant increase in urinary TCPy (115.6 µg) from the low to high dose. Therefore, the higher dose did not result in increased absorption when compared to the lower dose (i.e., percutaneous penetration rate was constant.) CPF clearance was not complete by 120 hours, therefore CPF or TCPy was likely retained in the skin and/or various body compartments. The elimination $T_{½}$ was 41 h indicating that repeated occupational exposure may result in accumulation of CPF and/or its metabolites.

II.B.3. PBPK-PD Model

Risk assessment of CPF is benefited from the use of the physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) model developed initially by Timchalk et al. (2002a); Timchalk et al. (2002b). The model generated PoD values based on 10% inhibition of RBC AChE after an acute (single day, 24 hr) or steady-state (21-d) exposure of CPF. When a steady-state has

occurred then the same inhibition is expected to continue for longer durations as shown in chronic animal studies. The model has undergone numerous revisions (Poet et al., 2003; Timchalk et al., 2007; Timchalk and Poet, 2008; Lowe et al., 2009; Smith et al., 2011; Poet et al., 2014; Smith et al., 2014; Poet, 2015; Poet et al., 2017a) to include such parameters as human life-stage (age related change of physiology and metabolism), pregnancy-related changes, as well as multi-route/variation (inhalation, oral, dermal). The data were judged to be acceptable for modeling because of completeness as well as having the best concordance for RBC AChE and BuChE inhibition and TCPy biomarkers for oral, dermal and inhalation routes of exposure(Timchalk and Poet, 2008; Poet et al., 2014). Note that some parameters are obtained by use of animal data.

III.B.4. PBPK-PD Model Predicts Life-Stage-Related Inter-individuality and Susceptibility to CPF

There are four main publications and one registrant submitted article that describe the development of the PBPK-PD model currently used in this risk assessment. All versions of the model have been validated, reviewed by outside experts, published in peer reviewed journals and externally reviewed by PBPK model experts. The models and their critical findings are described below:

Smith et al. (2011). Smith and colleagues investigated the age-dependent (life-stage) metabolism of CPF in human tissues. This model included CPF and CPF-oxon metabolism and TCPy metabolite disposition as well as carboxyesterase and plasma ChE inhibition. Metabolism was quantified by use of 20 samples of pediatric human microsomes (13-day to 6-month (n = 7), 6-month to 2-years (n = 6), and 2 to 12-years (n = 7)). Microsomes were cryopreserved and prepared by XenoTech, LLC (Lenexa, KS) according to standardized protocols[2]. Liver microsomal samples were procured from subject aged3 days to 75 years in order to optimize population distributions (e.g., to include potential sensitive individuals) but not compromise central tendency. Plasma samples (20 total) included pediatric 3-day to 6-month (n=5), 6-month to 2-year (n = 6), and 2- to 12-year (n = 4) age groups, along with five adult samples (age 16-43 years). **Microsomal Activity**: Metabolic activity in microsomes for the four main P450s associated with CPF metabolism (CYP1A2, 3A4/5, 2B6, and 2C19) was characterized (Sams et al., 2000; Tang et al., 2001; Buratti et al., 2003; Mutch and Williams, 2004; Sams et al., 2004; Foxenberg et al., 2011). Three P450 enzymes (CYP2B6, 2C19, and 3A4) had different age-related expression. **CYP2B6** occurred in 64% of fetal samples and had a 2-fold rise from birth to 1 month (variability = 25-fold). The high variability was likely due to individual metabolic

[2] XenoTech LLC, https://www.xenotech.com/company; Rewerts, C., Maciej Czerwinski, M. and Loewen, G. personal communication). Human livers were flash cryopreserved as is done for the purpose of organ transplant prior to microsome preparation (https://www.xenotech.com/products/subcellular-fractions/human/liver/microsomes). The stability of microsomes obtained from human livers has been documented over 10 years, with little effect in metabolic activity over multiple freeze-thaws during that time span. Utilization of microsomes derived from human tissues is described and recommended in the Federal Food and Drug Administration Guidance for Industry Drug Interaction Studies — Study Design, Data Analysis (FDA, 2012).

regulation and genetic polymorphisms (Croom et al., 2009). **CYP2C19** in newborns was 15% of adult values but increased in a linear fashion up to 5 months; at age 10 the values were similar to adults (21-fold variation) (Koukouritaki et al., 2004). In addition, CYP2C19 showed high, non-age-related variability (62-fold). **CYP3A4** was previously characterized as having low gene expression in infants, but by age 6-12 months it had increased to within 50% of adult levels (Blake et al., 2005). The activity levels increased beyond adult levels in late infancy and then decreased to adult levels over time (Blake et al., 2005). The late infancy surge could be explained by increasing CPF desulfuration and dearylation (CYP3A4 is involved in both reactions) product formation for both reactions (CPF-oxon and TCPy, respectively) without changing the product ratios. **Activity in Plasma:** Plasma samples were phenotyped for PON1 status and frequencies of PON1 [glycine (Gln; Q allele) to arginine (Arg; R allele)] genetic phenotypes were 0.5, 0.4, and 0.1 for QQ, QR, and RR phenotypes, respectively. Results showed that plasma PON1 metabolism of CPF-oxon had an age-related increase. This is in agreement with other studies reporting lower PON1 in newborns compared with adults (Cole et al., 2003; Holland et al., 2006). The difference was 26-32% lower for PON1 activity in newborns, depending on the phenotype, when compared to children at age 7, where levels were within 4% of adult PON1 activity (Huen et al., 2010). In the current study, CPF-oxon was metabolized at adult levels by age 10, based on plasma volume.

Smith et al. (2014). This study provided a description of human life-stage changes in a PBPK-PD model utilizing the measured parameters from Smith et al. (2011). Physiology and pharmacodynamic parameters relating to production of CPF-oxon and changes in activities of AChE, BuChE, and carboxylesterase in brain, diaphragm, liver, lungs, plasma, and RBCs were model inputs. Adipose and lipid compartments were added (Figures 5 and 6) to simulate the age-related variability in changes to body weight, organ volumes, and metabolism, after oral exposure to CPF. Parametric distribution was simulated for each metabolic parameter (means and coefficients of variation [CV] determined) by quantitatively integrating each age-dependent CPF and CPF-oxon metabolic parameter to represent a typical person. The descriptors for these age-dependent changes were obtained from controlled human CPF exposure studies for comparison to the model predictions (Nolan et al., 1987; Kisicki et al., 1999; Timchalk et al., 2002a; US EPA, 2014a). A sensitivity analysis was performed to pin-point the most critical parameters for estimating 10% RBC AChE inhibition after a simulated oral dose of 3 µg/kg CPF in 6 month old and 30 year old humans (Smith et al., 2014). Sensitivity endpoints also included TCPy in blood and urine, CPF in blood, and plasma ChE inhibition. Initially all parameters were fixed and the model was run to determine a baseline of variability. Then, systematically, each parameter was individually varied by ±1% until all parameters had been tested to determine which of the 120 parameters was the most sensitive to variation. Sensitivity coefficients (distribution of change in peak RBC AChEI ÷ change in parameter) were calculated for each parameter. Small parameter changes were ~1%. Greater changes meant a > 1% change in predicted RBC AChE inhibition. Values near zero meant that AChEI was not affected by that parameter. Modeled data were subsequently validated by findings in human dosing studies (Nolan et al., 1982; Nolan et al., 1984; Kisicki et al., 1999).

At doses $\geq$ 0.6 mg/kg, CPF was predicted to be lower and CPF-oxon higher in children compared to adults due to CPF metabolism-based body weight and liver/body weight differences. At $\geq$0.6 mg/kg the increases in CPF-oxon in children predicted by the model may be

due to the CPF-oxon levels overwhelming the metabolic capacity in plasma (Smith et al., 2011). However at <0.6 mg/kg, CPF-oxon is lower in children than adults due to increased metabolism in children at that exposure. Pharmacokinetic differences in metabolism and distribution are influenced by age-related body fat content because CPF is lipophilic and adults have more fat than 6 month old infants (~2-fold). Higher body fat can translate to lower CPF metabolism, altered distribution results, and increased half-life of CPF in adult blood to twice that of infants.

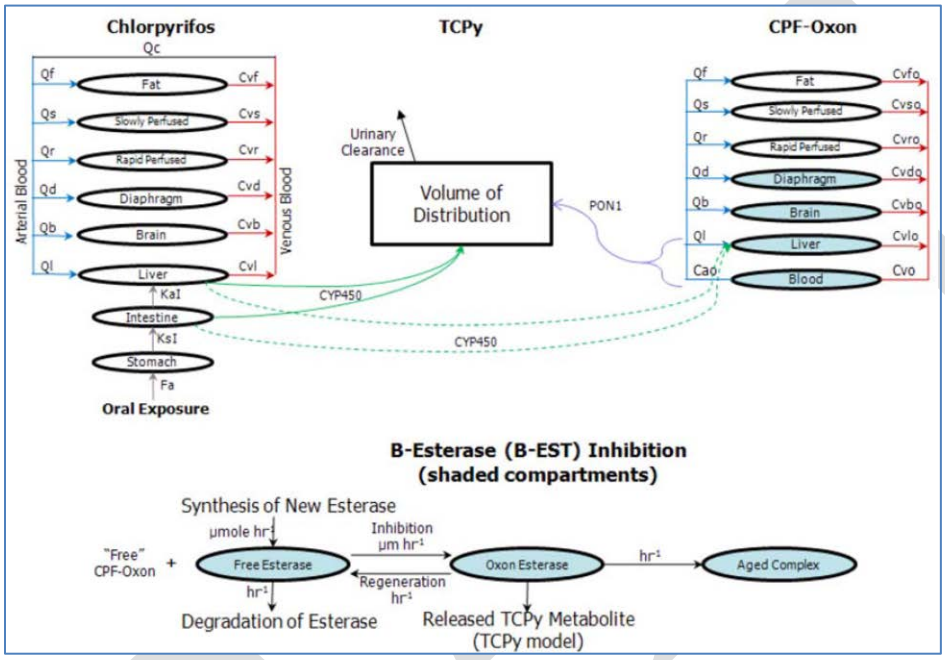


Figure 5. PBPK-PD Model Structure (typical adult)

The shaded compartments denote tissues which contain B-esterases (BuChE, CES: bottom panel). Tissue volumes and enzyme activities (Vmax) change with age based on liver and/or blood compartmental growth (Smith et al., 2014).

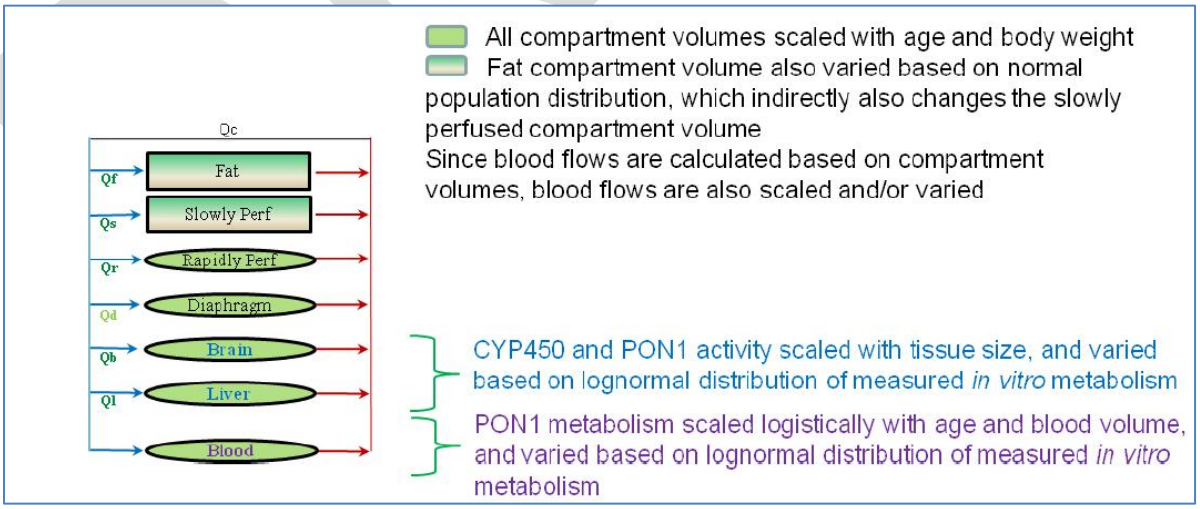


Figure 6. Schematic of Age and Body Weight Dependences in PBPK-PD model

Compartment volumes and blood flows vary with age and body weight. *In vivo* metabolic rates are scaled based on tissue size (measured *in vitro* values scaled to describe brain, blood, and liver metabolism); in blood, PON1 metabolism of oxon is dependent on blood volume and age (Smith et al., 2014).

Poet et al. (2014), Poet (2015). Poet and colleagues developed a multi-route (oral, dermal, and inhalation) PBPK-PD model for CPF and CPF-oxon metabolism. The oral life-stage model (Smith et al., 2014) served as a basis for optimizing metabolic rate constants and tissue growth in both humans and rats to apply to the multi-route model. Human metabolic data was collected from volunteers (7 males and non-pregnant females aged 21-55) who were exposed to Empire*20 insecticide (0.5% CPF in water) used to treat apartment carpet (Vaccaro et al., 1993). Two carpet treatments were done with four subjects in apartment #1 and three different subjects in apartment #2. After exposure, each volunteer (dressed in T-shirt and shorts) crawled, rolled, or laid on the carpet for 4 hours to simulate how a child might behave on the carpet in an apartment. Air exposure of CPF was also measured on the floor where most activity occurred (cassette filters backed by a Chromosorb tube 15 in were placed near each volunteer). Air samples from Apartment #1 had a time weighted average (TWA) of 11.4 mg/m^3. The TWA from Apartment #2 was 5.53 mg/m^3. Data from the cassettes were added to the model to estimate human exposure. An acute rat CPF (aerosolized) inhalation study provided parameters for the PBPK-PD modeled route (Hotchkiss et al., 2013). The authors note that the *in vivo* results for critical metabolic parameters (plasma CPF and CPF-oxon; TCPy concentration in urine; plasma, RBC and brain AChE inhibition) compared well with those predicted for humans in the PBPK-PD route. The authors go one to state that due to the low vapor-pressure of CPF, inhalation exposure is expected to be low and that based on modeled data, 23% of inhaled CPF (aerosol) in humans would be deposited in the alveolar region of the lung. The model assumes that CPF aerosol deposited in the nasal passages and upper and lower airways eventually reaches he liver. Therefore, liver metabolic activity (100% absorption) was used for the inhalation route (Corbo et al., 1989; Dahl and Hadley, 1991; Sarkar, 1992; Gerde et al., 1998; Song et al., 2004). Exhalation was included in the model, but is predicted to be near zero. B-esterases were included, but not PON1 (no lung data available).

For dermal exposure to CPF, the hands of each volunteer were rinsed 3 times in 250 ml of 0.008 dioctyl sodium sulfosuccinate soap. Hand surface area for adults is approximately 4% of the body and the rest of the body surface area (minus the part covered by T-shirt and shorts) is 66%. It was assumed in the study that the main parts of the body were subjected to the same dose. The normalized dermal dose was calculated for each individual's exposure based on body surface area (as calculated from their body weight), specific dermal absorption, and measured air sampling data. Nolan et al. (1984) showed that after a 5 mg/kg CPF dermal treatment in human volunteers, there was a 5-fold lower plasma ChE inhibition when compared to a 0.5 mg/kg oral dose. This information along with the TCPy measurements indicated that dermal absorption on the lower arm was 1.3% CPF over a 12-24 hour period, compared with almost 100% absorption via the oral route. Griffin et al. (1999) estimated that dermal absorption was 1% based on metabolites detected in urine. Data from the volunteer carpet study were used to validate the PBPK-PD model for the dermal route of CPF (Poet, 2015). Note that some parameters are obtained by use of animal data but as shown in Table 3, below, there was concordance between human and rat data for 4 major biomarkers. Using animal data in designing a PBPK model is

standard procedure. Parameters can be scaled to humans by use of body weights, blood flow, and other pharmacokinetic measurements.

Table 3. Data Concordance and Completeness for PBPK-PD Model Validation

Route	Pharmacokinetic (PK) Biomarkers					Cholinesterase Biomarkers			
	Blood CPF	Blood Oxon	Blood TCPy	Urine TCPy		Plasma	RBC	Diaphragm/ lung	Brain
ORAL						**ORAL**			
Rat Data	X	X	X	X		X	X	X	X
Human Data	X	X	X	X		X	X	--	--
INHALATION						**INHALATION**			
Rat Data	X	X	X	X		X	X	X	X
Human Data	--	--	--	--		--	--	--	--
DERMAL						**DERMAL**			
Rat Data	--	--	--	X		X	--	--	X
Human Data	X	--	X	X		X	X	--	--

[a]- "X" indicates measured data in rat and human for PBPK-PK validation
"—" indicates no data
Yellow highlighted area indicates measured data that was the most complete and showed the best concordance (rat and human) for RBC AChE and BuChE/plasma ChE inhibition and TCPy biomarkers for oral, and dermal routes of exposure (data from Poet et al. (2014); Timchalk and Poet (2008).

Poet et al. (2017a). Poet and colleagues built on previous versions of the PBPK-PD model to provide simulations of CPF and CPF-oxon metabolism after oral exposure in infants and adults and in pregnant and non-pregnant females. Modifications to the life-stage PBPK-PD model (Smith et al., 2011; Smith et al., 2014) included growth during pregnancy (metabolism, uterine, placental and fetal compartments; changes in slowly perfused and fat compartments; and, changes in blood such as increasing blood volume; decreasing hematocrit; increased lipids, triglycerides, cholesterol). The inter-individual differences in a parameter due to body composition and metabolic activity define variability while uncertainty is from model assumptions, extrapolations, or experimental data interpretation. Of the 120-160 parameters tested, sixteen were identified as having the greatest impact on AChE inhibition, accounting for >95% of total inter-individual variation (Table 4). Monte Carlo analyses were performed using the means and the coefficients of variance of the 16 distributions from the raw data from Smith et al. (2011)to generate 1000 simulated infants (6 months) and adults. These simulated subgroups were exposed to 0.3 mg/kg/day for one or 5 days to assess RBC AChE inhibition. Single dose tests were performed with 3000 simulated infants or adults. Degree of variability defined the most sensitive parameters based on the raw data and the sensitivity analyses from Smith et al (2014).

Table 4. Sixteen Main Parameters Considered in the PBPK-PD Model Design

Hepatic CYP activation of CPF-CPF-oxon	Total blood volume	RBC AChE degradation rate	Transfer rate of CPF or oxon from stomach to intestine
Hepatic PON1 CPF-oxon detoxification TCPy	Hepatic blood flow	RBC AChE degradation rate	Liver volume
PON1 CPF-oxon detoxification to TCPy in plasma	RBC AChE inhibition rate	Intestinal CYP CPF-oxon bioactivation	Hepatic carboxyl basal activity rate
Hepatic PON1 CPF-oxon detoxification to TCPy	Hematocrit	Intestinal CYP detoxification to TCPy	Hepatic carboxyl reactivation rate

After testing the 16 most sensitive parameters, four were identified as having the greatest impact on RBC AChE inhibition (Table 5). Bioactivation and detoxification had the greatest impact on RBC AChE, including physiology and non-metabolism parameters.

The liver microsome reactions were:

1) CYP450 activation of CPF to CPF-oxon
2) CYP450 detoxification of CPF-oxon to TCPy
3) PON1 detoxification of CPF-oxon to TCPy

The plasma reaction was:

PON1 detoxification of CPF-oxon to TCPy

The raw data from Smith et al. (2011) characterizing the two CYP450 reactions and two liver PON1 reactions were from 30 individuals. In order to characterize the impacts of small sample sizes on the means and coefficients of variance on the bioactivation and detoxification parameters (listed above), a parametric bootstrap methodology was applied. The bootstrap technique can increase the variability beyond that of the measured population samples (Table 5). Raw data was used in the PBPK-PD model to generate means and coefficients of variance for the major subpopulations (infants, men and women, non-pregnant and pregnant women) by Monte Carlo distributions (built into the model). These data for 1000 individuals were bootstrapped (resampled) 20 times (1000 individuals, 20 bootstraps = 20000 individuals) to maximize the initial small sample size and increase the variability of the critical parameters. The width of the dose-response showed that the doses eliciting 10% RBC AChE inhibition ranged from 0.08-2.4 mg/kg/d for CPF and from 0.03-0.9 mg/kg/d for CPF oxon. The bootstrap method resulted in a range of 3.5 (CYP450 to oxon) to 10-fold (plasma PON1 in adults) wider (Table 5) than the raw data (Smith et al., 2011). The predicted values were about twice the range reported for maternal (8.5-fold) and infant (34-fold) PON1 in plasma (Huen et al. (2012). According to Ginsberg et al. (2009), the intra-genotypic variability in activity due to the PON1 192 polymorphism in activity was 15-fold for CPF which is similar to that of all ages(Smith et al., 2011). The PBPK-PD model exceeds the range of CPF allotype variability by about 2-fold beyond the projected (measured) range for PON1 based on Ginsberg et al. (2009). It exceeds the measured PON1 activity values by a maximum of 10-fold when compared to the measured values from Smith et al. (2011). Table 5 summarizes the data for the 4 metabolism-related parameters and the comparative variability of raw data, parametrically distributed data (Monte Carlo), and bootstrapped/Monte Carlo distributions.

Table 5. Ratios of the Maximum to Minimum Value in the Raw Data and Bootstrap Model Simulations for the Critical Enzyme Activities

Parameter	**CYP450 to TCPy**	**CYP450 to Oxon**	**Hepatic PON1[a]**	**Plasma PON1[a]**
Range in raw *in vitro* data[b]	12	28	10/11[c]	6/16[c]
Range in parametric distribution[d]	26	34	33	33
Range in 20 parametric bootstraps[e]	74	98	58	58
Ratio[f]	1:6.1	1:3.5	~ 1:5.2	1:3.6/9.6

a -Values for PON1 in liver & plasma assumed to be correlated and thus have the same variation (Poet et al., 2017a)

b- Data based on Smith et al. (2011).
c- Smith et al. (2011): Hepatic PON1 Ratios Vmax (nmol/min/mg microsomal protein) = 10 (age 0.04-2 yr) and 11 all ages (0.04 to 75); Plasma PON1: Ratio Vmax (nmol/min/ml plasma) = 6 (age 0.01-2 yr) and 16 all ages (0.01 to 46)
d- Data based on Smith et al. (2014).
e- Data based on Poet et al. (2017a).
f- Ratio of raw data range to range in 20 parametric bootstraps.

Impact of Variability: Ninety percent of all summed model variability (global sensitivity) has parameters with a sensitivity coefficient of 0.3. Of the 160 model parameters, 20 have sensitivity coefficients of $\geq$0.1, accounting for more than 95% of all the local sensitivity. The remaining parameters showed almost no impact on modeled predictions. The critical parameters related to inter-individual variation in RBC AChE were for clearance of CPF and CPF-oxon.

Impact of Parameter Uncertainties: A Monte Carlo program was used to calculate Data Derived Extrapolation Factors (DDEF) for acute oral exposures for the following sub-populations: general population of adult males and females, non-pregnant females, pregnant females (8^{th} month; 3^{rd} trimester was determined to be most sensitive median pregnant females based on 10% RBC AChE inhibition), and infants 6 months of age. DDEF calculated for the above populations were designed to replace default uncertainty factors with quantitative intraspecies physiological and biochemical determinations.

$$DDEF_{HD} = PoD_{H} \div PoD_{SH}$$

PoD_H is the oral dose (ED_{50}) resulting in 10% RBC AChE inhibition for the median individual from a simulated population and PoD_{SH} is the oral dose (ED_{10}) resulting in 10% RBC AChEI for the 1^{st} percentile). The Monte Carlo program simulations allowed the researchers to evaluate the inter-individual variation of RBC AChE inhibition. DDEFs were very similar for CPF for males and females (3.4), infants (3.6), non-pregnant female (3.4) and pregnant female (2.9). For CPF-oxon the DDEF for males and females (1.8) was similar to infants (2.1); the other groups were not measured.

The range of PoDs (ED_{10}) for all populations was 0.39-0.52 mg/kg/d. Pregnant females had an ED_{10} that was 20% lower (0.39 mg/kg/d; most sensitive population) than that of non-pregnant females and adult men. A time course for pregnancy or for young life stages could not be performed but the model was adjusted based on data from the open literature on pregnancy-related changes in maternal metabolism and physiology. Changes in P450 CYPs relating to CPF and CPF-oxon metabolism showed 33% increased bioactivation and 25% decrease in detoxification over the course of pregnancy. PON1 in plasma and liver was decreased by 7% by week 26 of pregnancy. The simulated median for 10% RBC AChE inhibition in pregnant women was at doses of 3-20% less than nonpregnant women; however variability was also less in pregnant women. Pregnant women were only slightly more sensitive to CPF exposure than non-pregnant women; however at the 10th percentile the values were very similar. This may be due to changes in physiology or biochemistry during gestation. Poet et al. (2017a) have shown that inter-individual variability could decrease in pregnant women by increased CPF to CPF-oxon and decreased detoxification to TCPy metabolite. Due to pregnancy, the increased plasma lipids

could decrease the partitioning of CPF from blood to tissues and decrease intraspecies variability in metabolic clearance.

III.B.5. US EPA use of the PBPK Model to Simulate CPF Exposures

In 2016, US EPA developed a PBPK model to simulate CPF concentrations in human blood(US EPA, 2016b). PBPK exposure data were estimated from US EPA standard operating procedures (SOP) for indoor crack and crevice/hard surface use of CPF for the same time frame as the initial Columbia CCCEH Cohort study (1998-2004) (see footnote 1). These data were used in forward dosimetry to model blood levels of CPF in the pregnant women and newborn cord blood. It was assumed that biological responses are equivalent based on equal tissue doses (not equal external exposures). Biomarker data (CPF measurements in cord blood) from the Columbia CCEH Cohort were used as an *in vivo* standard for comparisons with predicted PBPK values (US EPA, 2016b). A benchmark dose analysis (linear regression) applied measured decrements in the working memory index (WMI measured by the Wechsler Intelligence Scale for Children WISC-IV) from children who were exposed to CPF *in utero* versus CPF measured in cord blood in newborns (Rauh et al., 2011). At the 1% change in WMI, the BMDL was close to the limit of detection (LOD) of 0.5-1.0 pg CPF/gram cord blood which introduced a great deal of uncertainty. However at the 3-5% change in WMI the CPF residues in cord blood were near the 6.17 pg CPF/g blood levels that are more closely associated with neurodevelopmental effects (Rauh et al., 2006; Rauh et al., 2015). A BMDL representing a 2% decrease in WMI was associated with an internal dose of 2.16 pg/g CPF in cord blood. Columbia Cohort publications did not report frequency of CPF exposure or timing in terms of maternal or cord blood sampling. Therefore forward dosimetry was used with PBPK modeling to compare the values for CPF in cord blood to predicted values from presumptive exposure scenarios and a known sequence of exposure/sampling parameters. The PBPK model was not used for the determination of a PoD, only for prediction of blood concentrations from likely exposure scenarios. A time-course for CPF concentrations in blood was simulated based on likely exposure scenarios and presumptive time between exposure and blood sampling (~4 hours to 2 days).

II.C. Acute and Short-Term Toxicity

The profile of acute CPF toxicity has been extensively described and reported by others (US EPA, 2007; Eaton et al., 2008; Testai et al., 2010; US EPA, 2011b; US EPA, 2014a). Severe poisoning in humans causes neurotoxic effects such as slurred speech, tremors, ataxia, convulsions, depression of respiratory and circulatory centers, which may culminate in coma and possibly death (Ecobichon, 2001). The following profile of acute toxicity for CPF consists of Health Effects Test Guideline studies submitted to HHA by registrants (see Appendix 1) as well as open literature studies that were considered by the current authors to be relevant and well-performed. Acute exposure to toxic levels of CPF results in the typical signs and symptoms of cholinergic toxicity: salivation, lacrimation, urination and defecation. The oral, dermal and inhalation LD_{50}, dermal and eye irritation, dermal sensitization, and acute delayed neurotoxicity studies using technical CPF and that were required for registration were submitted by the registrant (Table 6). Oral and dermal effects in the rat were primarily rated as Category II. Inhalation effects were rated Category II/III. Rabbits were not sensitive to CPF when applied dermally, however they did exhibit slight to moderate eye irritation. CPF did not cause dermal irritation, dermal sensitization, or acute delayed neurotoxicity.

Table 6. Acute Toxicity Studies for Technical Grade Chlorpyrifos

Study Type	Species	Result	Category	Reference [a]
Oral LD_{50}	Rat	223 mg/kg (M/F)	II	1*
	Rat	221 mg/kg (M) 144 mg/kg (F)	II	2*
Dermal LD_{50}	Rat	202	II	3*
	Rabbit	>5000 mg/kg (M/F)	IV	4*
	Rabbit	>2000 mg/kg (M/F)	IV	5*
Inhalation LC_{50}	Rat	> 4.07 mg/l (M) 2.89 (2.01 - 4.16) mg/l (F)	III	6*
	Rat	> 14 ppm (0.22 mg/l) M/F	II	7*
Primary Eye Irritation	Rabbit	Slight irritation (resolved within 24 hrs)	IV	8*
	Rabbit	Mild irritation	III	9*
Primary Dermal Irritation	Rabbit	Mild irritation (resolved within 7 days)	IV	10*
Dermal Sensitization	Guinea pig	Not sensitizing	NA	11*
Acute Delayed Neurotoxicity	Hen	No delayed neurotoxicity or other effects at HDT	NOEL>100 mg/kg/d	12*

[a] References: 1.Stebbins (1996b); 2. Nissimov and Nyska (1984b); 3.US EPA (2007); 4.Stebbins (1996a); 5. Nissimov and Nyska (1984a); 6. Buch (1980); 7. Landry et al. (1986); 8. Stebbins (1996e); 9.Buch and Gardner (1980); 10.Stebbins (1996d);11.Stebbins (1996c); 12. Rowe et al. (1978)
*The study was acceptable to HHA based on FIFRA guidelines.

The studies summarized in Table 7 are comprised of acute oral, dermal, or inhalation exposure to rats, mice, and rabbits during gestation, as neonates (pre-weaning), or as adults, as well as exposures to humans in order to compare AChE-related effects. Treatments are comprised of a single dosing or up to 10 days dosing by gavage, subcutaneous injection, dermal, or inhalation exposure. Study descriptions are found in greater detail in several sources (US EPA, 2007; US EPA, 2011b; US EPA, 2014a); See also Appendix 1 of this document). Findings from some of the open literature studies are described below.

Table 7. ChE Inhibition with Acute or Short Term (~2 week) Exposure to CPF and the Respective NOELs and LOELs

Species	Exposure	Cholinesterase Inhibition	NOEL mg/kg/d	LOEL mg/kg/d	Ref [a]
Oral Gavage or Subcutaneous Treatment to Pup/Neonate/Adult					
Rat SD M/F	Gavage c.o. or milk[b] PND 11	At 6-8 hr: ↓Plasma ChE, ↓RBC AChE ↓Brain AChE	Plasma: 0.5 RBC: 0.5 Brain 2.0	Plasma: 2.0 RBC: 2.0 Brain: 5.0	1
Rat SD M/F	Gavage c.o. PND 11-21	At 10 days 6 hr: ↓Plasma ChE ↓RBC AChE ↓Brain AChE	Plasma: 0.1 RBC: 0.1 Brain: 0.5	Plasma: 0.5 RBC: 0.5 Brain: 1.0	1
Rat SD M/F	Gavage c.o. PND 10-16[c]	At: 4 hr PND 16: ↓Plasma ChE ↓Brain AChE	Plasma: -- Brain: --	Plasma: 1.0 Brain: 1.0	2
Rat SD M/F	Gavage c.o. PND 10-16 [c]	At 4, 12, 24, & 48 hr PND 16: ↓Plasma ChE ↓Brain AChE	**Brain:** --	Brain: 1.0 (lowest dose tested)	3
Rat SD M/F	Gavage c.o. PND 10-16 [c]	At 4-10 hr PND 16: ↓Plasma ChE ↓Brain AChE	Plasma: – Brain: 0.5	Plasma: 0.5 Brain: 1.0	4
Rat SD M/F	Gavage c.o. PND 10-16 [c]	At 12 hr PND 16: ↓Bain AChE	Brain: 0.75	Brain: 1.0	5

Species	Exposure	Cholinesterase Inhibition	NOEL mg/kg/d	LOEL mg/kg/d	Ref [a]
Rat SD M/F	Gavage c.o. PND 10-16 [c]	At 12 hr PND 16: ↓Brain AChE	Brain: 0.75	Brain: 1.0	6
Rat M	Gavage c.o. PND 17	At 4 hr: ↓Whole blood AChE ↓Brain AChE	$BMDL_{10}$ [d] Blood: 0.43 Brain: 1.54	BMD_{10} [c] Blood: 0.62 Brain: 1.89	7
Rat SD M/F	Gavage c.o. Single treatment: PND 5, 12, 17	PND 5, 12, 17 at 3, 6 & 24 hr, respectively: ↓Plasma ChE, ↓RBC AChE ↓Brain AChE	RBC: -- Plasma: -- Brain: --	Plasma: 1.0 RBC: 1.0 Brain : 1.0	8
Rat SD M/F	Gavage c.o. PND 1-6 Tested PND 4, 7, 12	All time points: ↓Brain AChE	Brain: --	Brain: 1.5	9
Rat SD M/F	Gavage c.o. PND 1-21, 1-5, 6-13, 14-21	At 6 hr-9d PND 6, 12, 22, 30: ↓Brain AChE	Brain: --	Brain: 1.5	10
Rat SD M/F	Gavage c.o. PND 1-4 or 1-8	At 4 hr: PND 1-4: ↓Brain AChE	Brain: --	Brain: 1.0	11
Rat SD M/F PND 7 (neonate) PND 21 Adult 90d	Gavage Peanut Oil **Acute:** PND 7, 21 or 90 **Repeated**: 14d starting PND 7 or 90	All ages: 1 or 14 d at 4 hr post dose: ↓Plasma ChE ↓RBC AChE ↓Brain	Neonate acute: Plasma: 1.5 RBC: 0.75 Brain: 1.5 Neonate repeated: Plasma: 0.75 RBC: 0.75 Brain: 0.75 Adult acute: Plasma: 1.5 RBC: 0.75 Brain: ≥15 Adult repeated: Plasma: 0.45 RBC: 0.15 Brain: 1.5	Neonate acute: Plasma: 4.5 RBC: 1.5 Brain: 4.5 Neonate repeated: Plasma: 1.5 RBC: 1.5 Brain: 1.5 Adult acute: Plasma: 4.5 RBC: 1.5 Brain: ≥15 Adult repeated: Plasma: 0.75 RBC: 0.45 Brain: 4.5	12
Rat ? M/F	s.c. DMSO (1 ml/kg) PND 1-4	At 24 hr:↓Brainstem AChE	Brain: --	Brain: 1.0	13
Rat SD M/F	s.c. DMSO (1 ml/kg) PND 1 (1 dose only)	At 2 hr:↓Brainstem, cerebellum & forebrain AChE	Brain: --	Brain: 1.0	14
Mouse NMRI Pup M	Gavage 1:10 egg lecithin + peanut oil PND 10	↓ Brain AChE (only tested at 5.0 mg/kg/d)	Brain: <5.0 (only dose level for AChE)	Brain: 5.0	15
Oral Gavage or Subcutaneous Treatment to Dams During Gestation (Including DNT)					
Rat SD F	Gavage c.o. GD 6-PND 10 Test GD 20, PND 1,5 & 11	Dam GD 20 (24 hrs): ↓Plasma ChE, ↓RBC AChE ↓Brain AChE Pup: ↓Plasma ChE, ↓RBC AChE ↓ Brain AChE	Dam: Plasma: -- RBC: 0.3 Brain: 0.3 Pup: Plasma: 1.0 RBC: 1.0 Brain: 1.0	Dam: Plasma: RBC: 0.3 Brain: 1.0 Pup: Plasma: 5.0 RBC: 5.0 Brain: 5.0	16
Rat F-344 F	Gavage c.o. GD 6-15	At GD 21: ↓ Plasma ChE ↓RBC AChE	Dam: Plasma: 0.1 RBC: 0.1	Dam: Plasma: 3.0 RBC: 3.0	*17
Rat CD F	Gavage c.o. GD 6-15	At GD 20:↓ Plasma ChE	Plasma: --	Plasma: 0.5	*18
Rat Crl:CD7(SD) BR VAF/Plus F	Gavage c.o. GD6-LD 11	LD 22: ↓Plasma ChE, ↓RBC AChE ↓ Brain AChE	Dam: Plasma: -- RBC: -- Brain: 0.3	Dam: Plasma: 0.3 RBC: 0.3 Brain: 1.0	*19

Species	Exposure	Cholinesterase Inhibition	NOEL mg/kg/d	LOEL mg/kg/d	Ref [a]
Rat SD F	Gavage c.o. GD6-20	GD 20: ↓Plasma ChE, ↓RBC AChE ↓ Brain AChE	Dam: Plasma: -- RBC: -- Brain: 0.3	Dam: Plasma: 0.3 RBC: 0.3 Brain: 1.0	20
Mouse CF-1 F	Gavage cottonsee oil GD 6-15	At GD 18: ↓ Plasma ChE ↓RBC AChE	P0: Plasma: 0.1 RBC: 0.1	P0: Plasma: 1.0 RBC: 1.0	*21
Rabbit HY/CR-NZW F	Gavage c.o. GD 7-19	At GD 17d:↓ Plasma ChE	Dam: Plasma --	Dam: Plasma 2.5	*22
Rat SD M/F	s.c. DMSO (1 ml/kg) GD 9-12 or GD 17-20	At GD 21: ↓Brainstem & forebrain AChE	Brain: -- Only 1 dose level	Brain: 5.0	23
Adult Treatment					
Rat SD M/F	Gavage c.o. 10 d	At 6-8 hr D 10:↓Plasma ChE ↓Brain AChE	Plasma: 0.1 RBC: 0.1 Brain: 0.5	Plasma: 0.5 RBC: 0.5 Brain: 1.0	1
Rat SD F	Gavage c.o. Single dosing	At 8 hr: ↓Plasma ChE, ↓RBC AChE ↓Brain AChE	Adult: Plasma 0.5 RBC: 0.5 Brain: 2.0	Adult: Plasma: 2.0 RBC: 2.0 Brain: 10	1
Mouse C57Bl/6J M	s.c. DMSO (1 ml/kg); 1d or 5d	At 3-24 hr 5 injections: ↓Brain AChE	Brain: --	Brain: 5.0	24
Human M	1 dose (methylene chloride on a 0.5-g lactose tablet)	At 1-30 d: No significant effect on Plasma ChE	Plasma: --	Plasma: >0.5 (Only 1 dose level)	25
Human M/F	Powder in gelatin capsule[e]	At 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours post dose. ↓RBC AChE (1 subject)	RBC: 1.0	RBC: 2.0	26
Dermal Treatment					
Rat F344 F	Dermal c.o. 6 hr/d 4d	↓Plasma ChE ↓RBC AChE	Plasma: 1.0 RBC: 1.0	Plasma: 10.0 RBC: 10.0	27
Human M	1 exposure; dissolved in methylene chloride	No significant effect on Plasma ChE	Plasma: --	**Plasma:** >5 (Only 1 dose level)[f]	28
Inhalation Treatment (mg/m^3)					
Rat Crl:CD (SD) M/F	Aerosol Nose Only; 2-6 hrs	↓Plasma ChE ↓RBC AChE ↓Brain AChE	Plasma: – RBC: 3.7 Brain: 22.1	Plasma: 3.7 RBC: 12.9 Brain: 53.5	29
Rat CD(SD): Crl F	Vapor Nose Only; single dose	No significant effects on Plasma ChE, RBC or Brain AChE	Plasma: – RBC: -- Brain: --	Plasma: >0.254 RBC: >0.254 Brain: >0.254	30
Rat F-344 M/F	Vapor Nose only or Whole Body 6 hr	↓Plasma ChE in whole body exposure (attributed to oral ingestion or dermal exposure)	Plasma: 50.1	Plasma: 100.2	31

[a] References: 1. Marty et al. (2012), Marty and Andrus (2010); 2. Carr *et al.* (2011); 3. Carr et al. (2013); 4. Carr *et al.* (2014); Carr *et al.* (2015a); 5. Carr *et al.* 2015; 6. Carr 2017; 7. Moser et al. (2006); 8. Timchalk et al. (2006); 9. Betancourt and Carr (2004); 10. Richardson and Chambers (2005); 11. Guo-Ross et al. (2007); 12. Zheng et al. (2000); 13. Song et al. (1997); 14. Dam et al. (2000); 15. Mattsson *et al.* (2000a); 16. Ouellette et al. (1983); 17. Rubin et al. (1987a); 18. Hoberman (1998); 19. Maurissen et al. (2000); 20. Deacon et al. (1979); 21. Rubin et al. (1987b); 22. Qiao et al. (2002); 23. Speed et al. (2012); 25. Nolan et al. (1984); 26. Kisicki et al. (1999); 27. Calhoun and Johnson (1988); 28. Nolan et al. (1982); Griffin et al. (1999); 29. Hotchkiss et al. (2010); 30. Hotchkiss et al. (2013); 31. Landry *et al.* (1986b).

[b] Milk and corn oil (c.o.) results were the same for males and females except brain AChE with milk: NOEL: 2.0 M and 0.5 F

[c] Time of greatest post-natal brain development (PND 10-16)

[d] BMD and BMDL calculated by (US EPA, 2011a)
[e] Human volunteers treated at 0.5, 1.0 and 2.0 mg/kg CPF
[f] Reported as internal dose by (Hotchkiss et al., 2010)
* The study was acceptable to HHA based on FIFRA guidelines.
No NOEL denoted by –

II.D. Subchronic Toxicity

A number of acceptable Health Effects Test guideline subchronic studies are available for CPF as shown in Table 7, above. Table 8 focuses on NOELs and LOELs for plasma, RBC, and brain ChE inhibition in rats, mice, and dogs after oral, dermal, or inhalation exposure. Table 9 reports subchronic overt (non-ChE) effects in some of the same studies described in Table 7 (detailed in Appendix 1).

Table 8. AChE Inhibition with Subchronic Exposure to Chlorpyrifos and Respective NOELs and LOELs

Species	Exposure	Cholinesterase Inhibition	NOEL mg/kg/d	LOEL mg/kg/d	Ref [a]
Oral					
Rat F-344 M/F	Diet 13 Weeks	↓ Plasma ChE	Plasma: 0.1	Plasma: 1.0	1*
Rat SD M/F	Diet 2-Generation Reproduction	↓ Plasma ChE ↓ RBC AChE	Plasma: 0.1 RBC: 0.1	Plasma: 1.0 RBC: 1.0	2*
Rat Long-Evans F	Gavage c.o. 4 weeks	↓ Plasma ChE ↓ RBC AChE ↓ Brain AChE	Plasma: -- RBC: -- Brain: --	Plasma: 1.0 RBC: 1.0 Brain: 1.0	3*
Rat SD F	Diet 28 d	↓ RBC AChE ↓ Brain AChE	RBC: -- Brain: 0.4	RBC: 0.4 Brain: 2.0	4*
Rat Wistar M	Gavage c.o. 90 days	↓ Plasma ChE ↓ Brain AChE	Plasma: -- Brain: 1.3	Plasma: 1.3 Brain: 3.26	5
Beagle Dog M/F	Diet 6 weeks	↓ RBC AChE	RBC: --	RBC: 0.5	6
Dermal					
Rat F-344 M	21d, 6hr/d, 5d/wk	No effects	--	>5	7
Mice Balb/c M Adult (150 d) Pup (18 d)	4 hr/d, 2 weeks: 1 dose level administered on the tail	Pup/Adult: ↓ Plasma ChE	Pup/Adult: Plasma: --	Plasma: Pup/Adult: 101 Only 1 dose	8
Inhalation (mg/m^3)					
Rat F-344	Vapor, whole body	↓Plasma ChE	Plasma: 50	Plasma: 86	9*
Rat F-344 M/F	Vapor, Nose-only; 6 hr/d, 5d/wk, 13 weeks	No RBC, plasma, or brain ChE inhibition	--	>0.295	10
Rat F-344 M/F	Vapor, Nose-only; 6 hr/d, 5 d/wk, 13 wk	↓Plasma ChE	Plasma: 0.14 RBC: -- Brain: --	Plasma: 0.28 RBC: >0.28 Brain: >0.28	11

[a] References: 1. Szabo et al. (1988); 2. Breslin et al. (1991); 3. Maurissen et al. (1996); 4. Boverhof et al. (2010); 5. Wang et al. (2014); 6. Marable et al. (2001); 7. Calhoun and Johnson (1988); 8. Krishnan et al. (2012); 9. Landry et al. (1986a); 10.Corley et al. (1986); 11. Newton (1988)
*The study was acceptable to HHA based on FIFRA guidelines.
AChE: acetyl cholinesterase; RBC: red blood cell
No NOEL denoted by –

Table 9. Overt Effects with Subchronic Exposure to Chlorpyrifos and Respective NOELs and LOELs

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref [a]
Oral					
Rat SD M/F	Diet 2-Generation Reproduction	Parental:↑ vacuolation in the adrenal zona fasciculata, altered tinctorial properties in this tissue. Pup: ↓pup weights & pup survival	Parent/Pup: 1.0	Parent/Pup: 5.0	1*
Rat F-344 M/F	Diet 13 Week Neurotoxicity	↑ clinical signs, ↑FOB, motor activity effects	1.0	5.0	2*
Rat Long-Evans F	Gavage Corn Oil 4 weeks	↑miosis & clinical signs; motor slowing and/or ↓ motivation (↑"actual total delay", ↑ "void trials", ↓numbers of nose-pokes/trial).	1.0	3.0	3*
Rat SD F	Diet 28 d Immunotoxicity assay	↓absolute & relative spleen & thymus weights; ↑anti-SRBC assay effects [b]	0.4	2.0	4*
Dermal					
Rat F-344 M/F	21 day dermal	No overt effects	5	LOEL>5	5
Inhalation [c]					
Rat -344 M/F	Aerosol, Nose-only; 6 hr/d, 5 d/wk, 13 wk	No overt effects	--	>0.286 mg/m^3	6

[a]References: 1. Breslin et al. (1991); 2. Shankar et al. (1993); 3. Maurissen (1996); 4. Boverhof et al. (2010); 5. Calhoun and Johnson (1988); 6. Newton (1988)

[b] The Boverhof et al. (2010)females (10/dose) showed that the hematology parameters were not affected by CPF at any dose. The anti-SRBC IgM serum titers were less at 2 and 10 mg/kg/d (not dose-related manner; i.e., the titers for 2 and 10 mg/kg groups were 36 and 59% of the control group, respectively); considered equivocal based on the range of variability demonstrated in the control group values and the lack of a clear dose-response. Other parameters (spleen and thymus weights, white blood cell differential counts) did not indicate any suppression of immunopotency.

[c]- No subchronic inhalation studies with reported overt effects.

* The study was acceptable to HHA based on FIFRA guidelines

No NOEL denoted by –

II.E. Chronic Toxicity/Carcinogenicity

II.E.1. Animal Carcinogenicity

A number of acceptable Health Effects Test guideline chronic studies submitted by the registrant are available for CPF as shown below. Table 10 focuses on NOELs and LOELs plasma, RBC, and brain AChE in rats, mice, and dogs after oral exposure. Table 11 reports chronic overt (non-AChE) effects. There was no significant increase in tumors with any of these long-term studies. These studies are more fully described in the HHA Summary of Toxicology Data (Appendix 1). CPF is not considered to be a carcinogen.

Table 10. ChE Inhibition with Chronic Exposure to Chlorpyrifos and the Respective NOELs and LOELs

Species	Exposure	Cholinesterase Inhibition	NOEL mg/kg/d	LOEL mg/kg/d	Ref [b]
Oral [a]					
Rat F-344 M/F	Diet 2 yr	↓ Plasma ChE ↓ RBC AChE ↓ Brain AChE	Plasma: 0.1 RBC: 0.1 Brain: 1.0	Plasma 1.0 RBC: 1.0 Brain: 10	1*
Rat F-344M/F	Diet 2 yr	↓ Plasma ChE ↓ RBC AChE ↓ Brain AChE	Plasma: 0.2 RBC: 0.2 Brain: 5.0	Plasma: 5 RBC: 5 Brain: 100	2*
Dog Beagle M/F	Diet 2 yr	↓ Plasma ChE ↓ RBC AChE ↓ Brain AChE	Plasma: 0.01 RBC: 0.03 Brain: 1.0	Plasma: 0.03 RBC: 0.1 Brain 3.0	3*
Mouse CD-1	Diet 79 wks	↓ Plasma ChE ↓ RBC AChE ↓ Brain AChE	Plasma: -- RBC: 0.9 Brain: 9.1	Plasma: 0.9 RBC: 9.1 Brain: 43.9	4*

[a] No chronic dermal or inhalation studies
[b] References: 1. Young and Grandjean (1988); 2. Crown (1990); 3.McCollister et al. (1971); 4. Gur (1992)
* The study was acceptable to HHA based on FIFRA guidelines.
No NOEL denoted by –

Table 11. Overt Effects with Chronic Exposure to Chlorpyrifos and the Respective NOELs and LOELs

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref [b]
Oral [a]					
Rat F-344 M/F	Diet 2 yr	↓body weight; perineal yellow; vacuolation of the adrenal zona fasciculate; ↑diffuse retinal degeneration	1.0	10	1*
Rat F-344 M/F	Diet 2 yr	↓body weight; diffuse retinal atrophy & cataracts	1.25	50	2*
Dog Beagle M/F	Diet 2 yr	No systemic or non-ChE effects	--	LOEL> 61.7	3*
Mouse CD-1 M/F	Diet 79 wks	↓body weight, food & water consumption; ↑clinical signs; ↑Hepatocytic fatty vacuolation: centrilobular, Ulcerative dermatitis; Keratitis, panophthalmitis or endophthalmitis; accumulation of alveolar macrophages in lungs & septal thickening; bulbourethral gland cystic dilatation	0.78	7.9	4*

[a] No chronic dermal or inhalation studies
[b] References: 1. Young and Grandjean (1988); 2. Crown (1990); 3. McCollister et al. (1971); 4. Gur (1992)
* The study was acceptable to HHA based on FIFRA guidelines.
No NOEL denoted by --

II.E.1. Human Carcinogenicity

The Agricultural Health Study was conducted between 1993-1997 to investigate occupational pesticide exposure among farmers and commercial pesticide applicators and risk of cancer and other chronic diseases (Lee et al., 2004). The design was to examine risk factors for specific diseases (e.g., lung cancer) and then to focus on risk to subgroups with specific exposures. Participants were from Iowa and North Carolina and were characterized as <40 to ≥60 years

(n=57,311). Study enrollees completed questionnaires and cohort members were matched to cancer registries in Iowa and North Carolina and the National Death Index annually for case identification from 1993 through 2001. Questionnaires were self-administered to obtain comprehensive exposure data for 22 pesticides and ever/never use data for 28 pesticides, along with personal protective equipment used, pesticide application methods, pesticide mixing status, equipment repair methods, smoking history, alcohol consumption, history of cancer in first-degree relatives, and basic demographic data. The study participants also had a take-home questionnaire with questions having to do with detailed occupational and medical history and diet. The take-home questionnaire was returned by 24,671 pesticide applicators (43%). Most of the cohort (>60%) were less than 50 years of age and more than 50% were never smokers.

Lee et al. (2004) focused on CPF since it is widely used nationally. Questionnaire answers indicated that among the subjects with complete exposure data, 22,181 (41%) had used CPF. In order to evaluate a potential association between CPF exposure and cancer incidence, a Poisson regression analysis was used (after adjustment for potential confounders; two-sided). A CPF association for both lung cancer incidence and CPF intensity-weighted exposure days was reported. Subjects in the highest quartile for life-time of exposure-days (>56), along with adjustments for other pesticide exposures and demographics had a relative risk for lung cancer of 2.18 times (95% confidence interval: 1.31 to 3.64) that of subjects who were not exposed. The increased lung cancer risk was primarily limited to smokers who received the longest exposure (>56 days). In addition, the CPF-exposed applicators used this pesticide for an average of 6.6 years and for 9.4 days/year, with the highest quartile at >56 days (224 mean; 116 median) lifetime exposure-days. The authors defined pesticide applicators who used CPF as "exposed" and those who did not use CPF as "nonexposed." However, since CPF is so widely used, there is the possibility that these subjects received CPF exposure by non-occupational routes, leading to potential misclassification of exposure. In addition, product formulation and application methods for CPF have changed since the 1997 completion of the study, so the author caution that the data should be interpreted with that fact in mind (Lee et al., 2004).

Lee et al. (2007), used results from the Agricultural Health Study cohort of pesticide applicators described in Lee et al. (2004) to investigate incidence of rectal cancer associated with pesticide exposure. There were 50 pesticides which were analyzed for associations with colorectal cancer and occupational exposures. Pesticide applicators with no prior history of colorectal cancer (n=56,813) were included. Cancer registries showed that 212 colon and 93 rectal cancers were diagnosed in this cohort from the time of enrollment (1993) to 2002. CPF had an exposure response for rectal cancer at a 2.7-fold (95% confidence interval: 1.2–6.4) higher risk at the highest exposures (highest quartile of exposure days: >56). The study authors indicated that a potential confounder is subject recall bias associated with CPF use. Since there were 50 pesticides with multiple comparisons in this study, some statistically significant associations may have been due to chance alone. The authors suggest that further research is warranted.

Waddell et al. (2001) conducted a study with pooled data from three population-based case-control studies conducted in Kansas, Nebraska, Iowa, and Minnesota. They investigated the potential for an association between organophosphates (OP) use and non- Hodgkin's lymphoma (NHL) among white male farmers. Iowa/Minnesota subjects (≥age 30; n=780) with diagnosed NHL between 1981 and 1983. Nebraska subjects with NHL were diagnosed between 1983 and 1986 (≥age 21 years; n = 227). Telephone interviews were performed to obtain data on demo

graphics, medical conditions, family history of cancer, tobacco and alcohol use, occupation, agricultural practices, hobbies, and an abbreviated dietary history. The interview also involved detailed questions about agricultural practices, personal use of specific pesticides, years of use, days per year of use, protective practices, livestock and crops grown, and other farm-related activities. Persons who reported actual use of pesticides were considered to be exposed. The control subjects (n = 3379) were selected from the Health Care Financing Administration records. They were matched to living cases (≥65 years) by state, race, gender, 5-year age group, and vital status at the time of interview. The control subjects for the cases who were deceased were from state mortality records that were matched for year of death. There were 993 cases and 2918 controls who were actually interviewed Data were evaluated by calculating odds ratios (ORs) and 95% confidence intervals (CIs) by logistic regression analysis using a SAS program. Results among farmers showed 158 cases and 279 controls who had used OPs, including 117 direct and 41 proxy respondents among cases and 224 direct and 55 proxy respondents among controls (proxy majority = spouses). CPF OP was not mentioned in this study, although several others were (diazinon, malathion, and terbufos).

II.F. Genotoxicity

CPF is not mutagenic in bacteria (Simmon et al., 1977; Bruce and Zempel, 1986a; Bruce and Zempel, 1986b) or mammalian cells (Mendrala, 1985), but did cause slight DNA damage in yeast (Simmon et al., 1977). Mitotic recombination-gene conversion in yeast exposed to a 5% concentration of CPF for 4 hours, with and without metabolic activation was studied. No individual data were presented and without this the significance of the effect cannot be evaluated however, the possible genotoxic effect must be noted.

CPF did not result in DNA damage in human embryo fibroblasts or rat primary hepatocytes *in vitro* (Simmon et al., 1977; Mendrala and Dryzga, 1986). CPF was not clastogenic in the mouse micronucleus test *in vivo* (McClintock and Gollapudi, 1989). CPF did not induce unscheduled DNA synthesis in isolated rat hepatocytes (Mendrala, 1985). Mehta et al. (2008) treated male Wistar rats with CPF for 1, 2 or 3 days at 50 or 100 mg/kg/d or for 90 days at 1.12 or 2.24 mg/kg/d. Results showed increased DNA damage in liver and brain at all doses tested in all dosing regimens, especially at acute levels. This is likely because the treatment levels were above the maximally tolerated dose and excessively high, particularly at the acute levels. Therefore, it was not surprising that some form of cytotoxicity was noted. This study had several deficiencies, including the lack of cytotoxicity data, there was no positive control, the animals were treated intramuscularly, and data analysis was based on data point rather than number of animals. Rahman et al. (2002) tested CPF for the ability to induce *in vivo* genotoxic effect in leucocytes of Swiss albino mice using the single cell gel electrophoresis assay or comet assay. The mice were gavaged with CPF (0.28 to 8.96 mg/kg; no vehicle description; dosing schedule not described so single acute doses were assumed). Body weight and whole blood leukocytes were examined at 24, 48, 72, and 96 h. There was a dose-related increase in mean comet tail length, indicating DNA damage was observed at 24h post-treatment ($p<0.05$) with CPF in comparison to control. At 72 hours, all DNA effects were repaired except at > 4.48 mg/kg. By 96 h post-treatment, the mean comet tail length reached control levels indicating repair of the damaged DNA. This study had numerous deficiencies, including a lack of description of statistical analysis and no positive control.

II.G. Reproductive Toxicity

CPF (98.5% pure) was fed in the diet to Sprague-Dawley rats from premating through F_2 weaning (2 generations, 1 litter/generation) (Breslin et al., 1991). Concentrations were adjusted as needed to achieve exposures of 0, 0.1, 1.0, and 5.0 mg/kg/d. Treatment began approximately 10 and 12 weeks prior to breeding for the F_0 and F_1 adults, respectively. The ChE inhibition NOEL was 0.1 mg/kg/d based on decreased plasma and RBC AChE at 1.0 and 5.0 mg/kg/d (see Table 12). The parental NOEL was 1.0 mg/kg/d based on increased degree of vacuolation in zona fasciculata especially in males, as well as altered tinctorial properties in females. The reproductive NOEL was 1.0 mg/kg/d based on slightly reduced pup weights and slightly reduced pup survival at 5.0 mg/kg/d. There were no clinical signs specifically indicating cholinesterase inhibition. The reproductive findings at 5 mg/kg/d do not warrant a "possible adverse effects" designation, since brain cholinesterase levels were very markedly depressed at that dose level and all observed reproductive effects appeared to be due to failure of dams to nurture pups.

II.H. Developmental Toxicity

Table 12 summarizes acceptable Health Effects Test guideline CPF studies submitted by the registrant as well as open literature studies. All studies are detailed Appendix 1 as well as in the US EPA risk assessment documents (US EPA, 2007; US EPA, 2011a; US EPA, 2014a). The developmental studies reported below focus on overt effects and ChE inhibition in rat, mouse, and rabbit dams and fetuses after oral or dermal exposure of CPF to dams during gestation and in some cases to pups during the pre-weaning period. CPF was not teratogenic however; developmental delays (delayed ossification, decreased birth weight and lower crown-rump length) and increased implantation loss were observed at higher doses in rats, mice, and rabbits.

Table 12. Developmental Effects of CPF and the Respective NOELs and LOELs

Species	Exposure	Effects[a]	NOEL mg/kg/d	LOEL mg/kg/d	Ref [b]
Oral Gavage Treatment to Dams During Gestation (including DNT)					
Rat F-344	Gavage GD 6-15 Cottonseed o	**Dam:** Cholinergic signs, clinical signs, ↓ body enlarged adrenals **Fetus**: No developmental effects	Dam: 3.0 Fetus: 15	Dam: 15 Fetus: >15	1*
Rat CD	Gavage GD 6-15 Cottonseed o	**Dam:** Tremors, ↓ food consumption; ↓body weight **Fetus**: ↑post-implantation loss	Dam/Fetus: 2.5	Dam/Fetus: 15	2*
Mice CD-1	Gavage GD 6-15 Cottonseed o	**Dam**: Cholinergic signs, ↓ food and water consumption, ↓body weight gain **Fetus**: ↓live fetuses; ↓body weight; ↓crown-rump length; ↑delayed ossification in skull & sternabrae	Dam: 1.0 Fetus: 10	Dam: 10 Fetus: 25	3*
Rabbit HY/CR-NZW	Gavage GD 7-19 c.o.	**Dam:** ↓body weight gain **Fetus:** ↓body weight; ↓crown-rump length; ↑delayed ossification in 5th sternabrae & xiphisternum	Dam/Fetus: 81	Dam/Fetus: 140	4*
Dermal Treatment Pups and Adults					
Mice Balb/c M Adult (150 d) Pup (18 d)	4 hr/d, 2 weeks: 1 dose only	**Adult:** Dissolution of Nissl granules[c]; ↑GPAF[d] **Pup:** pyknosis in Purkinje neurons in cerebellum	Only 1 dose--	Pup/Adult: 101 Pup/Adult	5

[a] Effects on plasma, RBC and brain cholinesterase inhibition for these studies shown in Table 7 above.
[b] References: 1. Ouellette et al. (1983); 2. Rubin et al. (1987a); 3. Deacon et al. (1979); 4. Rubin et al. (1987b); 5. Krishnan et al. (2012)
[c] Nissl granules: free ribosomes in neuronal rough endoplasmic reticulum that are a site of protein synthesis.
[d] GPAF Glial fibrillary acidic protein, necessary for regulating astrocyte motility(Pekny et al., 1999) .
* The study was acceptable to HHA based on FIFRA guidelines.
No NOEL denoted –

Table 13. Effects of Chlorpyrifos on the Endocannabinoid System in Pre-Weaning Sprague-Dawley Rats

Dose	Endocannabinoid Effects[a]	NOEL mg/kg/d	LOEL mg/kg/d	Ref [b]
Oral Gavage Treatment to Pups/Neonates (Males and Females Gavaged with Corn Oil PND 10-16)				
1, 2.5, 5.0 mg/kg/d	↓Brain MAGL & FAAH activity ↓2-AG & AEA hydrolysis (4 hr termination)	MAGL: -- FAAH: -- AEA: -- 2-AG: --	MAGL: 1.0 FAAH: 1.0 AEA: 1.0 2-AG: 1.0	1
1.0, 2.5 or 5.0 mg/kg/d	↓Brain MAGL & FAAH at 4 hrs post-terminal dose ↓2-AG & AEA hydrolysis at 12 hrs post terminal dose	MAGL: -- FAAH: -- AEA: -- 2-AG: --	MAGL: 1.0 FAAH: 1.0 AEA: 1.0 2-AG: 1.0	2
0.5, 0.75 or 1.0 mg/kg/d	↓FAAH activity at 4 & 12h; ↑AEA	FAAH: -- AEA: --	FAAH : 0.5 AEA: 0.5	3
0.5, 0.75 or 1.0 mg/kg/d	↓Brain MAGL & FAAH activity ↓2-AG & AEA hydrolysis;	MAGL: 0.75 FAAH: -- AEA: -- 2-AG: 0.5	MAGL: 1.0 FAAH: 0.5 AEA: 0.5 2-AG: 0.75	4*
0.5, 0.75 or 1.0 mg/kg/d	↓MAGL ↓FAAH activity ↓2-AG hydrolysis, at 12 hr post terminal dose.	MAGL: 0.75 FAAH: -- 2-AG: 0.75	MAGL: 1.0 FAAH: 0.5 2-AG: 1.0	5

[a] Effects on plasma, RBC and brain cholinesterase inhibition for these studies shown in Table 7 above.
[b] References: 1. Carr et al. (2011); 2. Carr et al. (2013); 3. Carr et al. (2014); 4. Carr et al. (2015a); 5. Carr et al. 2017
* The study was acceptable to HHA based on FIFRA guidelines.
No NOEL denoted –
Abbreviations: AEA - anandamide; 2-AG - 2-arachidonoylglycerol; FAAH - fatty acid amide hydrolase; MAGL - monoacylglycerol lipase;

US EPA has not established a critical NOEL based on brain AChEI. Their critical acute PoDs in the 2011 and 2014 Preliminary and Revised Human Health Risk Assessments are based on 10% RBC AChEI. The critical PoD in the 2006 RED was based on plasma ChEI with a NOEL = 0.5 mg/kg/d. Table 14 compares RBC and brain AChEI in non-pregnant and pregnant rats (after 11 and 15 doses of CPF). The NOEL ($BMDL_{10}$) for brain AChE is at about 3-fold higher than RBC in non-pregnant animals and approximately 18-fold higher in pregnant animals.

Table 14. Comparison of RBC AChE and Brain AChE Inhibition in Rat Studies

Endpoint	Response	Comments
Repeated Dose ChEI - male and female rats (Hoberman, 1998; Mattsson et al., 1998; Maurissen et al., 2000; Marty and Andrus, 2010)	Female rats, 11 days (CCA) BMD10/BMDL10: RBC AChEI: 0.45/0.35 Brain AChEI: 1.03/0.95 mg/kg/d	Pregnant female rats more sensitive than non-pregnant female rats for RBC and Brain AChEI
	Female pregnant rats GD6-20; 15 days (DNT) BMD10/BMDL10: RBC AChEI: 0.06/0.03 mg/kg/d Brain AChEI: 0.65/0.54 mg/kg/d	RBC AChEI: 7.5-12 fold more sensitive Brain AChEI: 1.6-1.8 fold more sensitive

CCA: comparative cholinesterase study (Table from US EPA 2011a; page 25)

II.I. Behavior and Developmental Neurotoxicity

Studies that reported neurobehavioral and neurodevelopmental effects after CPF treatment included a developmental neurotoxicity study (DNT) submitted by the registrant, as well as published studies. These studies are detailed in the HHA Summary of Toxicology Data (Appendix 1), in the US EPA risk assessment documents (US EPA, 2007; US EPA, 2011a; US EPA, 2014a) and in a recent review of the neurodevelopmental effects of organophosphates (Lim and Bolstad, 2017). Table 15 focuses on neurobehavioral effects in pups that were treated with CPF postnatally and/or after rat or mouse pregnant dams were treated with CPF by oral gavage, diet, subcutaneous injection or dermally. Some citations overlap with those in Tables 7 and 13 but the focus in Tables 15 and 16 is specifically on neurobehavioral effects.

The studies were divided into two tables based on routes of exposure. Table 15 includes data with animals treated with CPF orally or dermally. HHA also reviewed studies employing routes of administration that mimic expected routes of exposure in humans, if they provide information pertinent to the selection of critical PoDs. The studies presented in Table 16 reported effects in animals treated with CPF by subcutaneous injection (s.c.). In some cases, dimethylsulfoxide (DMSO) was used as a vehicle for injection. At 1 ml/kg (standard DMSO vehicle concentration) DMSO did not have effects on brain AChE inhibition or neurotoxicity in rats (Whitney et al., 1995; Carr and Nail, 2008).

The most common neurodevelopmental outcomes observed in these studies were effects on cognition, motor control and social behavior. Qualitatively similar effects have been reported in the CPF epidemiology studies. Most animal studies in Table 15 and 16 were conducted with doses that also produced AChE inhibition at some time during the exposure. While the overall evidence indicates that CPF may cause neurodevelopmental effects, HHA identify few studies that included doses lower than 1 mg/kg/day, the threshold for ChE inhibition. These studies are summarized below.

Silva et al. (2017). Silva and colleagues investigated the effects on complex behaviors (particularly anxiety and depression) in Wistar rats exposed to CPF in utero. Pregnant dams (11-14/dose) received 7 consecutive daily doses of CPF (0.01, 0.1, 1 and 10 mg/kg/day) by oral gavage on gestation days 14–20. Controls received the vehicle only---Tween20 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 offspring were evaluated twice, during the infant-juvenile period (postnatal day [PND] 21) and in adulthood (PND70). Reproductive parameters---maternal body weight and weight gain, clinical signs of toxicity, gestation length, number of implants, post-implantation losses, average weight of offspring, offspring/mother 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 PND70. 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 determined in dams or pups. Gestational exposures to 10 mg/kg/day CPF resulted in reduced body weight gains in mothers during the treatment period. Maternal toxicity was not observed at lower doses. There were no clinical signs or effects on pregnancy that could be attributed to treatment. PND21 pups exposed in utero to 0.1 mg/kg/day showed anxiety-like behaviors, evident both in the statistically reduced times they spent in the open arms of the elevated plus-maze and in the increased locomotor activities detected in the open-field tests ($p < 0.05$ for both). Statistically significant effects were also observed at 1 and 10 mg/kg/day, though dose-dependent increases were not observed. There was no effect of CPF on depressive-like behavior as evaluated in the modified forced swimming test. PND70 animals did displayed neither anxiogenic nor motor activity behaviors. As with the PND21 animals, no changes in depressive behavior were detected in the modified forced swimming test. The authors concluded that CPF treatment during pregnancy induced anxiogenic behavior in pups at the end of lactation (PND21). As a result, they set the LOEL for neurodevelopmental effects at 0.1 mg/kg/day. The apparent absence of a dose-related exacerbation of this response above 0.1 mg/kg/day was unexplained, but was plausibly due to saturation of one or more of the many neural pathways unquestionably involved in regulation of complex behaviors such as these. For risk assessment purposes, the most important implication of this study is that the threshold for CPF-induced neurobehavioral effects in young rats following gestational exposure may be as much as 10-fold lower than the reported threshold of 1 mg/kg/day established for RBC AChE inhibition in adult rats

Lee et al. (2015). Male NMRI mice were treated CPF to investigate whether neurotoxicity occurs during rapid brain growth and maturation. A brain AChE inhibition group received CPF by gavage at 0 (20% fat emulsion/kg b.w. [1:10 egg lecithin + peanut oil]) and 5.0 mg/kg on PND 10 (n=4/dose) in a single treatment with assays performed at 1, 3, 6, 12, 24 or 36 hours post-dose. The vehicle was designed to simulate the fat content of mouse milk (~14%) in order facilitate the physiologically accurate absorption and distribution. Another group of males were treated with a single gavage dose of CPF at 0 and 5 mg/kg for protein analysis on PND 10. These mice were terminated at 24 hours or 4 months after exposure and the hippocampus and cerebral cortex were frozen (n=5-8/dose). A third group of mice were treated with CPF by gavage on PND 10 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). Results showed that brain AChE inhibition was minimal, even at 5.0 mg/kg (↓8–12% peak at 3 hrs post-dose). CaMKII and synaptophysin were decreased at 5.0 mg/kg 24 hours post-dose but was reversed at 4 months. These proteins are associated with a brain growth spurt in mice. Results of behavioral tests showed there were dose × time at 2 months of age for locomotion, rearing and total activity variables, respectively. Pairwise testing

between CPF-exposed and control groups showed a significant difference in these 3 variables at 5 mg/kg/d. Locomotion and rearing means were decreased at 1 and 5.0 mg/kg/d thus. The LOEL for behavioral effects in mice was 0.1 mg/kg based on.

Gomez-Gimenez et al. (2017). Pregnant Wistar rats (6/dose) were treated with CPF at 0, 0.1, 0.3 and 1.0 mg/kg/d GD 7-PND 21 using corn oil + sweet jelly as a dietary vehicle. The purpose of the study was to see if CPF effects are gender-related, observe effects on spatial learning after developmental exposure and if hippocampal neuroinflammation is associated with effects on spatial learning after CPF exposure during development. Pups were weaned PND 21 and were tested for spatial learning (Morris water maze, 8-arm radial maze) at 2-3 months of age. At 5-7 days after the behavioral tests, rats (7-12 males/dose/group; 5-10 females/dose/group) were terminated and the hippocampus was for proteins indicative of neuroinflammation (Iba-1, IL-4 and IL-10, IL-1β and TNF-α, GABA- α 1, GABA α 5 and GABA γ2, GluR1, GluR2, NR1, NR2A and NR2B). Results showed equivocal effects on escape latency in the Morris water maze (time to reach platform) at all doses in males and no effects on females on day 3 of testing. Males did not show a dose-response, however because 0.1 mg/kg/d showed the highest escape latency, while 0.3 and 1.0 mg/kg/d values were equivalent. Time spent in right quandrant on day 3 of testing was decreased in males at 1.0 mg/kg/d CPF 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/d and were equal to effects at 1.0 mg/kg/d. Females showed decreases at 1.0 mg/kg/d. Working errors (visits to arms already visited in the same trial) over the 5 days of testing were increased in males at 0.3 mg/kg/d, but again, were the same at 1.0 mg/kg/d; females were not statistically significantly affected. Learning index (#correct choice ÷ #errors for first entry into each arm) at day 4 were decreased in males at >0.3 mg/kg and were again the same value at the high dose. Females were statistically significantly increased at 1.0 mg/kg/d. It is difficult to interpret the meaning of this result. Males showed decreased IL10 at 1.0 mg/kg/d, while females had decreases at >0.3 mg/kg/d. Neuroinflammation was also equivocal since only one parameter (IL10) was positive out of 13 tested in both sexes. There was a definite difference in behavioral effects between males and females (males more affected). Since many of the results reported were equivocal for males, it would have been useful to see results from all testing days to see if effects were reversed. It would also have been useful to know how many pups/dose were tested in the behavioral studies. It is presumed based on the numbers used for neuroinflammatory protein tests. Most effects occurred at >0.3 mg/kg/d in males (discounting equivocal, non-dose-response effects), however there were effects to IL10 in females at 0.3 mg/kg/d. The LOEL for neuroinflammation is 0.1 mg/kg/d for both males and females.

Several studies from Carr's laboratory provided evidence for CPF-induced behavioral effects in young rats that may occur at doses lower than the threshold established for RBC AChE inhibition. The findings from these studies were presented in Section III.A.1., Acute and Short-Term Oral Toxicity and in Tables 13 and 15.

Table 15. Neurobehavioral Effects after Pre- and Postnatal Exposure to Chlorpyrifos

Dosing Period	ChE Inhibition	ChE Testing	Domain Affected [a]	Age of Behavior Testing	NOEL LOEL mg/kg/d				Ref [b]
					Plasma ChE	RBC AChE	Brain AChE	Behavior	
Oral Gavage to Sprague-Dawley Rat Pups/Neonates or to Fetuses *In Utero*									
Gavage c.o. PND 1-21 Dose regimen[c]	Brain AChE	PND 20, 30, 40, 50	↓ cognition (↓working & ↓reference memory:M; M more affected than F	PND 29-60	NA	NA	1.0 4.0	4 6.0	1
Gavage c.o. PND 1-21 Dose regimen[d]	Plasma ChE Brain AChE BuChE	PND 25, 30	↓ motor activity (line crosses) PND 25 & 30 No M/F difference	PND 25, 30	-- 1.0	NA	-- 1.0	1.0 3.0	2
Gavage c.o. PND 10-16 0.5, 0.75 & 1.0 mg/kg/d	Not tested	NA	↑ open field effects & ↑motor activity, (elevated plus maze, chasing crawling over/ under, play fighting, playing) No M/F difference	PND 25	NA	NA	NA	-- 0.5	3
Gavage c.o. PND 10-16 0.5, 0.75 & 1.0 mg/kg/d	Not tested	NA	↑anxiety & ↓sociability (↑time of emergence into illuminated area) No M/F difference	PND 25	NA	NA	NA	-- 0.5	4
Gavage c.o. PND 10-16 0.5, 0.75 & 1.0 mg/kg/d	Brain AChE	PND 16	↓anxiety; ↑sociability (↓time to emergence from a dark container to a novel aversive environment); No M/F difference	PND 25	NA	NA	0.75 1.0	-- 0.5	5
Gavage c.o. GD 6-LD 11 0.3, 1.0, 5.0 mg/kg/d	Dam Brain AChE	LD 22	↓ motor activity ↓ neuromotor function (↓latency to peak response for auditory startle habituation) ↓parietal cortex size; ↑hippocampal gyrus alterations; No M/F difference	PND 12-71	NA	NA	1.0 5.0	-- 1.0	6*
Oral Gavage to Wistar Rat Dams									
Gavage 10% Tween 20 in saline GD 14-20 0.01, 0.1, 1.0, 10 mg/kg/d	Not tested	NA	↑cognition (↓% time in open-arm of elevated plus maze); ↑motor activity (anxiogenic behavior) Only M tested	PND 21 and 70 by PND 70	NA	NA	NA	0.01 0.1	7
c.o. + sweet jelly in diet GD 7- PND 21 0.1, 0.3, 1.0 mg/kg/d	Not tested	NA	cognition (spatial reference errors ↑M, ↓F, working errors ↑M, learning index ↓M↑F); M more affected than F	2-3 months of age	NA	NA	NA	0.1 0.3	8
Oral Gavage to Mouse Dams									
CD-1 Gavage peanut oil GD 14-17 Only 1 dose: 6.0 mg/kg/d	Not tested	NA	F: ↑anxiety, emotion & social behavior (↑thigmotaxis; ↓ latency to enter in the dark compartment, ↑time in tunnel between sides in	PND 90	NA	NA	NA	1 dose 6.0	9

			light-dark box), 5HT system involvement[f]						
NMRI Gavage 1:10 egg lecithin + peanut oil[e] PND 10 0.1, 1.0, 5.0 mg/kg/d	Brain AChE	PND 10	↓ spontaneous movement in a novel home environment (↓motor activity; ↑rearing) Only M tested	PND 60 & 120	NA	NA	Only 1 dose tested 5.0	0.1 1.0	10
Dermal Treatment to Sprague-Dawley Dams During Gestation									
1 mg/kg/d in 70% ETOH) GD 4-20	Brain AChE	PND 90	↓basic neuromotor function (↓grip time M/F, ↓incline plane degrees; F) F more affected than M	PND 90	NA	NA	Only 1 dose 1.0	1 dose 1.0	11
Long-Evans Female Rat Adult Oral									
Gavage c.o. 4 week Cognitive Study 1, 3, 10 mg/kg/d	Plasma ChE RBC AChE Brain AChE	Day 21	motor slowing and/or ↓ motivation & memory (↑actual total delay, ↑ void trials, ↓number of nose-pokes/trial)[g]	Day 21 & Day 28	-- 1.0	-- 1.0	-- 1.0	3.0 10	12

[a] Parameters include neuropathology, brain weights, morphometrics, motor activity, body temperature, auditory startle response, delayed spatial alternation, ***assessments of choice, learning and working memory*** (T-maze for spontaneous alternation, radial arm water maze, 8-arm radial maze; passive/active avoidance of a specific event, rewarded behavior), ***locomotor activity*** (open field movements, maze challenges), ***neuromotor function*** (sensorimotor function; auditory startle: latency and magnitude; prepulse inhibition [reflex response]; fore- and hindlimb grip strength; degrees on an inclined plane), ***social behavior*** (sexual behavior, rearing, play-fighting, licking), ***socioagonistic behavior*** (fighting and attacking), ***balance coordination*** (negative geotaxis on an inclined plane), ***anxiety and risk taking*** (elevated plus maze, the open field test, and the light/dark choice test) and ***depressive behaviors*** (forced swim test).

[b] References: 1. Johnson *et al.* (2009); 2. Carr *et al.* (2001); 3.Carr *et al.* (2015a); 4. Mohammed *et al.* (2015); 5. Carr *et al.* (2015b); 6. Hoberman (1998); 7. Silva *et al.* (2017); 8. Gomez-Gimenez *et al.* (2017); 9. Venerosi *et al.* (2010); 10. Lee *et al.* (2015); 11. Abou-Donia *et al.* (2006); 12. Maurissen (1996); Table adapted in part from US EPA (2014a)

[c] Dosing regimen: 0 (c.o. vehicle), **low dose**: 1.0 mg/kg/d PND 1-20, **medium dose**: 1.0 mg/kg/d PND 1-5, 2.0 mg/kg/d PND 6-13, 4.0 mg/kg/d PND 14-20; **high dose**: 1.5 mg/kg/d PND 1-5, 3.0 mg/kg/d PND 6-13, 6.0 mg/kg/d PND 14-20.

[d] Dosing regimen: 0 (c.o. vehicle), **low dose**: 3.0 mg/kg every other day PND 1-21, **medium dose**: 3.0 mg/kg every other day PND 1-5 followed by 6.0 mg/kg/d every other day from PND 7-21; **high dose**: 3.0 mg/kg every other day PND 1-5, 6.0 mg/kg every other day PND 7-13, then 12 mg/kg every other day PND 15-21.

[e] Dosing regimen CPF at 0 (peanut oil), 3 or 6 mg/kg by gavage to dams GDs 15 to 18 by intraoral gavage; Postnatal treatment, CPF at 0, 1 or 3 mg/kg sc to prenatally treated pups from PNDs 11 to 14. Each litter assigned to one prenatal treatment (vehicle, 3 or 6 mg/kg), one male and one female were randomly assigned to vehicle (Veh), one male and one female to CPF 1 mg/kg (CPF1), and one male and one female to CPF 3 mg/kg (CPF3). Total = 9 treatment groups: preVeh-postVeh, preCPF3-postVeh, preCPF6-postVeh, preVeh-postCPF1, preCPF3-postCPF1, preCPF6-postCPF1, preVeh-postCPF3, preCPF3-postCPF3 and preCPF6-postCPF3.

[f] 5HT: serotonin or 5-hydroxytryptamine a monoamine neurotransmitter contributing to feelings of well-being, memory and cognition

[g] "actual total delay" (time of first lever press to press of the correct choice lever); "void trials" delays longer than set criteria; "nosepokes/trial" memory retention.

Table 16. Neurobehavioral Effects after Subcutaneous Pre- and Postnatal Injections of Chlorpyrifos

Dosing Period	ChE Inhibition	ChE Testing	Domain Affected [a]	Age of Behavior Testing	NOEL LOEL mg/kg/d				Ref [a]
					Plasma ChE	RBC AChE	Brain AChE	Behavior	
Subcutaneous Treatment to Male and Female Rat Pups									
Rat Long-Evans s.c. Peanut oil PND 11, 15 0.3, 7 mg/kg/d	Brain AChE	PND 11, 16, 28	↓ cognition (↑latencies to find platform in Morris water maze, ↓ time in training quadrant) No M/F difference	PND 7, 11, 15	NA	NA	O.3 7.0	-- 0.3	1
s.c. DMSO (1 ml'kg) PND 1-4; 1, 11-14 5 mg/kg	Brain AChE	PND 1, 11	↓ motor activity (M); neuromotor function (↓rearing PND 1-4 & ↑PND 11-14 (M); ↑righting reflex (F); ↓negative geotaxis (F)) No M/F difference	PND 3-4 (reflex righting),; Negative geotaxis[b], PND 5-8; PND 21, 30 (motor skills)	NA	NA	-- 1.0	-- 1.0	2
Rat SD Pup M/F s.c. DMSO (1 ml'kg) PND 1-4 1 mg/kg/d	Not tested	**NA**	↑motor activity (↑ center crossings in elevated plus maze, M.); ↓cognition (↑ radial arm maze working & reference memory errors, M; ↓working memory errors in radial arm maze, F) ↓anxiety (↑ open arm time in elevated plus maze);↓ chocolate milk preference (anhedonia)), M more affected than F	PND 52-53 & 64+	NA	NA	NA	1 dose tested 1.0	3
s.c. DMSO (conc not stated) 1 mg/kg/d PND 1-4 or 5 mg/kg/d PND 11-14	Not tested	NA	↓Spatial learning, memory (F) F more affected than F	T-maze spontaneous alternation & Figure-7 & locomotor activity: weeks 4–6; radial-arm; maze training weeks 14-17	NA	NA	NA	1 dose tested 1.0	4
Mouse Dam and Offspring									
CD-1 s.c. Peanut Oil PND 11-14 3 mg/kg/d Treated F mated PND 60	Not tested	NA	Pups: ↓Sociability F after giving birth: ↑anxiety & emotion (↓time to enter light side), ↑social behavior & maternal interaction (↑ latency to build nest, ↓latency to lick pups, ↓defensive; ↑digging) Pups:↑anxiety (↓motion in new cage) . No M/F difference	Pups: PND 40-45 After mating: PND 60; maternal behavior tested LD 1-7	NA	NA	NA	1 dose tested 3.0	5
Subcutaneous Treatment to Sprague-Dawley Rat Dams During Gestation and/or Pups									
s.c. DMSO (1 ml/kg) GD 9-12 1.0, 5.0 mg/kg/d	Not tested	NA	↑motor activity (↑ habituation, ↓ latencies in t-maze, ↑ center crosses in elevated plus maze); ↓cognition (↑radial arm maze working & reference memory errors) No M/F difference	PND 28-91	NA	NA	NA	1.0 5.0	6
s.c. injection DMSO (1 ml/kg) GD 17–20 1.0, 5.0 mg/kg/d	Not tested	**NA**	↑**motor activity** (↓t-maze latencies, ↓Fig 8 habituation, ↓radial arm latency); ↓cognition (↑radial arm maze working & Reference memory errors F) F more affected than M	PND 28-42, 56-91	NA	NA	NA	-- 1.0	7
Subcutaneous Treatment to Sprague-Dawley Rat Dams During Gestation and/or to their Pups									
CD-1 Pup F s.c. Peanut Oil GD 15-18 &	Not tested	NA	F Pups of dams treated 6.0 mg/kg/d GD 15-18: ↑social investigation, ↑vocalization; ↑motor activity & ↑exploring; F only	PND 120	NA	NA	NA	3.0 6.0	8

PND 11-14 3, 6 mg/kg/d									
CD-1 gavage peanut oil GD 15-18 (3 & 6 mg/kg/d) + s.c. peanut oil PND 1-14 (1 & 3 mg/kg/d)[c]	Pups only: Plasma Brain	24 hr post dose	**Dam**: ↓ Social behavior (↓licking, ↓sniffing; ↑crouching) **Pup (pre & post-natal treatment)**:↑ motor activity (↑crossing open field), ↓anxiety & emotion (↓head dips in +maze); ↑social behavior (↑attack response & offensive posture (M)) PN treatment: ↑%time in open arm (F); F affected more than M	PND 70, 75-80, 90, 120	-- 1.0	NA	6.0 >6.0	Dam: 3.0 6.0 Pup: 1.0 3.0	9
HS/lb s.c. DMSO (conc. not stated) GD 9-18 1, 3, 5, 10, 20 mg/kg/d	Not tested	NA	↓cognition (↓Morris water maze learning) No M/F difference	Pups PND 75	NA	NA	NA	-- 1.0	10
HS/lb s.c. DMSO (1 ml/kg) GD 9-18 3.0 mg/kg/d	Not tested	NA	↓cognition (↓Morris water maze learning) M/F data pooled	PND 80	NA	NA	NA	Only 1 dose tested 3.0	11
Swiss Webster Pup F s.c. DMSO (conc. not stated) GD 17-20	Not tested	NA	↓cognition (↓learning of food recognition & position) ; F only	PND 60-81	NA	NA	NA	-- 1.0	12
Swiss-CD-1 s.c. DMSO (conc. not stated) PND 1-4 PND 11-14 1, 3 mg/kg/d	Pup Plasma Brain	PND 4	↑motor activity (↑ activity at door opening in 2-chamber box (M)); ↓social behavior (↓self-grooming M/F); ↑agonistic behavior (M); M more affected than F	PND 25, 35-38, 38, 45, 60	-- 3.0	NA	>6.0	-- 1.0	13
ICR s.c. DMSO (conc?); GD 13-17 at 1, 5 mg/kg/d	Not tested	NA	↓ memory (T-maze delayed spatial alteration); M more affected than F	PND 45-60	NA	NA	NA	1.0 5.0	14

a References: 1. Jett et al. (2001); 2.Dam *et al.* (2000); 3. Aldridge et al. (2005a); (Aldridge et al., 2005b); 4. Levin *et al.* (2001); 5. Venerosi et al. (2008); 6. Icenogle et al. (2004); 7. Levin et al. (2002); 8. Venerosi et al. (2006); 9. Ricceri et al. (2006); 10. Billauer-Haimovitch et al. (2009); 11. Turgeman et al. (2011); 12. Haviland et al. (2010); 13. Ricceri et al. (2003); 14. Chen et al. 2012; Table adapted in part from US EPA (2014a).

b Negative geotaxis: ability to turn 180° on an inclined plane.

c Dosing regimen CPF at 0 (peanut oil), 3 or 6 mg/kg by gavage to dams GDs 15 to 18 by intraoral gavage; Postnatal treatment, CPF at 0, 1 or 3 mg/kg sc to prenatally treated pups from PNDs 11 to 14. Each litter assigned to one prenatal treatment (vehicle, 3 or 6 mg/kg), one male and one female were randomly assigned to vehicle (Veh), one male and one female to CPF 1 mg/kg (CPF1), and one male and one female to CPF 3 mg/kg (CPF3). Total = 9 treatment groups: preVeh-postVeh, preCPF3-postVeh, preCPF6-postVeh, preVeh-postCPF1, preCPF3-postCPF1, preCPF6-postCPF1, preVeh-postCPF3, preCPF3-postCPF3 and preCPF6-postCPF3.

No NOEL denoted "—"

* DMSO used as a vehicle at approximately 1 ml/kg. This dose is reported to be non-toxic in animal studies (Whitney *et al.*, 1995).

II.J. Immunotoxicity

CPF was administered in diet to female Sprague-Dawley rats (10/sex/group) at 0, 0.4, 2.0 and 10.0 mg/kg/d for 28 days (Boverhof et al., 2010). Another 10 females were dosed by intraperitoneal (i.p.) injection with 20 mg/kg/d of cyclophosphamide from day 24 through day 28 as the positive control group. No deaths occurred during the treatment period. There were no treatment-related effects on body weight or food consumption. The hematology parameters were not affected by the treatment. RBC AChE activity was reduced in a dose-related manner for all treatment groups. Brain AChE activity was significantly less than that of the controls at the 2 and

10 mg/kg treatment levels. The mean absolute and relative weights of the spleen and thymus were not affected by the treatment. The anti-SRBC IgM serum titers were reduced for the 2 and 10 mg/kg treatment groups. However, the effect was not manifested in a dose-related manner (i.e., the titers for 2 and 10 mg/kg groups were 36 and 59% of the control group, respectively). These results were judged to be equivocal based on the range of variability demonstrated in the control group values and the lack of a clear dose-response. Other parameters (spleen and thymus weights, white blood cell differential counts) did not indicate any suppression of immunopotency. The positive control was functional. The AChE NOEL was less than 0.4 mg/kg/d and the immunology NOEL was 0.4 mg/kg/d.

II.K. Epidemiological Studies Related to Neurodevelopmental Effects

There are several ongoing prospective cohort studies investigating the associations between environmental exposures during pregnancy or in early childhood and the effects on learning, development, and behavior. Many of these have included the evaluation of potential exposure to organophosphate pesticides, including chlorpyrifos.

II.K.1. Biomarkers of Human Chlorpyrifos Metabolism

Understanding the results of the epidemiological studies is helped by providing context for the variety of markers analyzed in these studies. For humans, metabolic activation of chlorpyrifos occurs predominantly in the liver while detoxification can take place in the liver or plasma (ATSDR, 1997; FAO/WHO, 1999). Metabolism is generally rapid and extensive, with the parent and/or the active metabolite found only in trace concentrations in blood or urine (ATSDR, 1997; FAO/WHO, 1999). The biological half-life for the major metabolite in humans following oral or dermal exposure was approximately 27 hours (Nolan et al., 1984) and chlorpyrifos metabolites are excreted primarily in the urine (ATSDR, 1997; FAO/WHO, 1999). The following table summarizes the main nonspecific metabolites of OP pesticides. See also Figure 4 earlier in this document.

Table 17. Specific and Nonspecific Urinary Metabolites of OP Pesticides in Humans

Pesticide	**Non-specific dialkyl phosphate (DAP) metabolites**			**Specific metabolites**
Chlorpyrifos	DEP	-	DETP	TCPy
Chlorpyrifos-Methyl	-	DMP	DMTP	TCPy
Diazinon	DEP	-	DETP	-
Oxydemeton methyl	-	DMP	DMTP	-
Methamidophos	-	DMP	DMTP	-

DAP - Dialkyl phosphate
DEP - Diethyl phosphate
DMP - Dimethyl phosphate
DETP - Diethyl thiophosphate
DMTP - Dimethyl thiophosphate
TCPy - 3,5,6-trichloro-2-pyridinol

Barr and Angerer (2006) succinctly categorized the biomarkers and environmental exposures for chlorpyrifos as follows:

- Biomarker of CPF Exposure: TCPy, DEP, DETP, CPF-oxon
- Biomarker of Effect: AChE inhibition
- Biomarker of Susceptibility: PON1 genotype/phenotype
- Primary route of environmental exposure: Diet
- Biologically active agent: CPF-oxon

Summaries of recent findings from major epidemiological cohorts as well as other independent studies are enumerated below.

II.K.2. Childhood Autism Risks from Genetics and the Environment: The CHARGE Study, The MIND Institute, University of California Davis Medical Center

The CHARGE study started in 2003 to investigate environmental causes and risk factors for autism and developmental delay. The CHARGE study has enrolled over 1600 participants and the pediatric participants either have either full autism spectrum disorder or developmental delay. Children in the study must be between 24-60 months of age when enrolled and have been born in California. The children are assessed for social, intellectual, and behavioral development. Questionnaires are designed to collect information about chemical use in the home, environmental exposures, medical history, diet, and alcohol and drug use both before and after birth.

Shelton et al. (2014) used data from the CHARGE study to determine whether mothers of children identified as having autism spectrum disorder or developmental delay lived near reported applications of certain agricultural pesticides (including carbamates, organophosphates, organochlorines, or pyrethroids) while pregnant with the affected children. Proximity to chlorpyrifos applications was independently assessed. Parents who completed the surveys were asked for all addresses where they lived going back to 3 months before conception. Participating children were given standardized tests to classify them as having autism spectrum disorder or developmental delay or if they were normally ("typical") developing for purposes of the study. The authors used information from the DPR 1997-2008 Pesticide Use Report (PUR) Database as a surrogate for actual exposures. Exposure levels (e.g., levels of parent compound or metabolites in blood, urine, or tissues) or durations were not measured in either the mothers during pregnancy or in the infants at birth or during the years of follow up.

Addresses of the cohort mothers were identified as being within 1.25 km, 1.5 km and 1.75 km of an agricultural pesticide application in the 3 months prior to conception through full-term delivery. The children evaluated in the cohort included 486 autism spectrum disorder cases, 168 developmental delay cases, and 316 cases that were normally developing. The study used Multinomial Logistic Regression to calculate odd ratio (OR) of autism spectrum, developmental delay, or typical development associated with residential location. The major findings were that children of mothers living near OP pesticide applications during the third trimester were at greater risk for autism spectrum disorder (60%). OP pesticide applications that occurred within 1.5 km of designated residences during the third trimester included documentation of use of 21 unique OP pesticides, including chlorpyrifos (20.7%), acephate (15.4%), and diazinon (14.5%). Researchers found a positive association between maternal proximity to chlorpyrifos applications

(1.5 km) in the second trimester and autism spectrum disorder (14% higher risk). In addition, the association between autism spectrum disorder and developmental delay and applications near residences during pregnancy decreased with increased distance from the application site. Altogether, the study concluded that when biological samples are unavailable, proximity to pesticides can serve as a proxy of potential exposure in the assessment of associations between environmental exposures and neurodevelopmental delay (Shelton and Hertz-Picciotto, 2015).

II.K.3. The Mount Sinai Children's Environmental Health Cohort, Children's Environmental Health Center, Icahn School of Medicine at Mount Sinai

From 1998 to 2002, the Mount Sinai Children's Environmental Health Study enrolled a multiethnic population of more than 400 pregnant women into a prospective study to investigate linkages between environmental exposures and impaired child cognitive development. All mothers gave birth at Mount Sinai Hospital in New York City between May 1998 and July 2001. They were screened and excluded for various potentially confounding birth parameters, including serious chronic diseases, a serious pregnancy complication that could affect fetal growth and development, and risky health behaviors including alcohol consumption in excess of two alcoholic beverages per day or illicit drug use. Children who were born with a congenital malformation or who were severely premature were also excluded.

The research team collected urine samples from the mothers during pregnancy and analyzed them for the evidence of metabolized pesticides. Questionnaires were administered to obtain information on characteristics such as environmental exposures, maternal smoking, and indoor pesticide use. The women participated in follow-up interviews when their children reached 12 months, 24 months, and 6 - 9 years of age. At 12 and 24 months, the children were assessed using the Bayley Scales of Infant Development for mental and psychomotor developmental indices. Between the ages of 6-9 years old, the children were given the Wechsler Intelligence Scale for Children 3rd or 4th version (WISC-III or WISC IV) with composite indices for Verbal Comprehension, Working Memory, Processing Speed, and Perceptual Reasoning, as well as Full Scale IQ.

The concentration of 3,5,6-trichloro-2-pyridinol (TCPy) and non-specific measures of OP pesticide exposure were measured in maternal urine collected during the 3rd trimester and in infant cord blood samples at birth. Berkowitz et al. (2003) measured TCPy concentrations in urine in 365 participating mothers. Forty-two percent of samples were above the limit of detection (LOD) of 12.0 µg/L and the median concentration adjusted for creatinine was 11.3 µg/g. The authors found no association between reported pesticide use or exposure in the questionnaire results and the quantitative urinary metabolite measurements (Berkowitz et al., 2003). The authors went on to assess the correlation between urinary pesticide metabolite concentrations, fetal growth measures, and metabolizing enzyme activity (paraoxonase-1, PON1). The authors found a significant positive trend between maternal paraoxonase activity and decreased head circumference among the offspring of mothers whose prenatal measures of TCPy were above the LOD (Berkowitz et al., 2004). When TCPy concentrations were removed from the equation, the trend remained for the association between decreased head circumference and PON1 activity, independent of any measure of pesticide exposure (Berkowitz et al., 2004). Associations between birthweight were also assessed. Wolff et al. (2007)found no significant association between diethylphosphate (DEP) concentrations and PON1 activity or the PON_{192}

genotype and decrements in birthweight. However, there was a 164 g deficit in birthweight between the extremes of interaction. That is, the slowest PON1 enzymatic activity and the highest total DEP concentrations were associated with the biggest decrements in birthweight, although none of the associations was significant (Wolff et al., 2007).

Researchers then considered the associations between concentrations of prenatal urinary metabolites and metabolites present at the time of birth and mental or psychomotor developmental indices, WICS-III or WISC-IV composite indices, Full Scale IQ, as well as with PON1 enzymatic activity levels and PON1 genotypes (Engel et al., 2011). Third-trimester maternal urine samples (n=360) were analyzed for OP metabolites and maternal blood samples were analyzed for PON1 activity and genotype. The Bayley Scales of Infant Development for mental development and psychomotor development were administered at approximately 12 months of age (n=200) and 24 months of age (n=276). There was no association between total diethylphosphate (DEP) metabolites and decreases in mental development indices at 12 months of age. There was no association between any OP urinary metabolite psychomotor development indices at 12 months of age. At 12 months, children of mothers with the $PON1_{192/QR/RR}$ genotype experienced a 2 point decline in the mental development index for each $\log_{10}$ unit increase in total DEP concentration in prenatal urine, although this effect also disappeared at 24 months. Increasing total DEP urinary metabolites were associated with slight decrements in Full Scale IQ, Perceptual Reasoning, and Working Memory assessed when the children were 6-9 years old, although the estimated effects were modest and imprecise. The overall results support the association of prenatal OP exposure and the presence of specific PON1 genotypes associated with slower catalytic activities with negative effects on cognitive development. However, the authors note that reconciling estimated effects when only using nonspecific urinary metabolites can be complicated when those metabolites derive from multiple parent compounds (Engel et al., 2011).

II.K.4. Mothers and Newborn Cohort, Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University

The Columbia Center for Children's Environmental Health (CCCEH, or Columbia) enrolled a sample of pregnant nonsmoking African-American and Dominican women between 18-35 years old residing in Washington Heights, Central Harlem, and the South Bronx, New York. The cohort started in 1997 to evaluate effects of prenatal exposure to ambient and indoor pollutants on birth outcomes, neurocognitive development, and procarcinogenic damage among a cohort of mother and newborns from minority communities in New York City (Whyatt et al., 2003). In 1998, the study began collecting information on prenatal pesticide use and exposure in response to growing concern of the extent of residential pesticide use (Whyatt et al., 2003). Ethnicity was self-identified and the women had registered at the OB/GYN clinics at NY Presbyterian Medical Center or Harlem Hospital by their 20^{th} week of pregnancy. The prospective cohort was designed to assess exposure to environmental contaminants and the effects on birth outcomes. The cohort lived in New York for more than one year before pregnancy and was screened for history of various potential confounders (drug abuse, diabetes, hypertension, or HIV infection). Potential exposure was measured as CPF in maternal blood collected within 1 day post-partum and fetal cord blood collected at delivery, as TCPy in maternal and fetal urine and meconium within 2 days of delivery, and via air concentrations collected by personal monitors during the third trimester of pregnancy (Perera et al., 2003; Whyatt et al., 2003). Participants responded to

questionnaires during the third trimester of pregnancy and then at follow-up assessments. The birth outcomes, delivery outcomes, and related medical information were also obtained for each participant. The cohort children were assessed for multiple measures of growth and development thought the years of follow-up, including an assessment of brain morphology between the approximate ages of 6 – 11. CPF was detected in 98% of maternal blood samples (mean = 7.1 pg/g) and 94% of cord blood samples (mean = 7.6 pg/g) (Perera et al., 2003)and the CPF concentrations in maternal (n = 263) and newborn (n=256) blood were highly correlated (r = 0.76) (Whyatt et al., 2004). The authors note that this shows CPF readily transfers from maternal to cord blood across the placenta. There was an association with CPF blood concentrations and decreased birthweight, which was significant in African-American mothers. CPF blood concentrations were associated with nonsignificant reductions in birth length in a subset of Dominican women. No associations were found between CPF blood concentrations at birth and head circumference (Perera et al., 2003). It is important to note that the association with CPF blood levels and reductions in birthweight and birth length were significant ($p = 0.008$ and 0.004, respectively) for infants born before January 1, 2001 (n=237) when compared to infants born after January 1, 2001 (n=77) (Whyatt et al., 2004). This likely reflects an overlap in subject recruitment with the US EPA restrictions on indoor chlorpyrifos use.

Air sampling was conducted for 2 consecutive days in the third trimester for mothers enrolled in the study from September 1998 through May 2001 (Whyatt et al., 2003). Indoor air concentrations ranged from 0.7 – 193 ng/m^3 CPF (Perera et al., 2003). Air concentrations collected < 1 month before delivery were highly correlated with maternal and cord blood CPF concentrations (Whyatt et al., 2003). However, there were no significant associations between OP pesticide air monitoring results and any birth outcomes (Whyatt et al., 2004).

Rauh and colleagues conducted a follow-up examination of the cohort children at 12, 24, and 36 months of age with the purpose of investigating the impact of prenatal CPF exposure on neurodevelopment and behavior (Rauh et al., 2006). Results showed that children categorized as highly exposed (maternal post-partum or cord blood levels > 6.17 pg CPF/g plasma) scored on average 6.5 points lower on the Bayley Psychomotor Development Index and 3.3 points lower on the Bayley Mental Development Index compared with those with lower CPF blood levels. Higher CPF blood levels were also significantly associated with attention problems, attention-deficit/hyperactivity disorder problems, and pervasive developmental disorder problems at 3 years of age (Rauh et al., 2006). The same cohort of children were again examined at 7 years old to estimate the long term effects prenatal CPF exposure on neurodevelopment using the Wechsler Intelligence Scale for Children – 4th Edition (WISC-IV) with composite indices for Verbal Comprehension, Working Memory, Processing Speed, and Perceptual Reasoning, as well as Full Scale IQ (Rauh et al., 2011). There were significant inverse correlations between CPF and Working Memory ($r = -0.21$; $p<0.0001$) and Full Scale IQ ($r = -0.13$; $p<0.02$), as well as a weak correlation between CPF and Perceptual Reasoning. There was a dose-effect relationship of CPF and log-transformed Working Memory and Full Scale IQ, with decreases of 2.8% and 1.4%, respectively, for each standard deviation (± 4.61 pg CPF/g cord blood plasma) increase in CPF exposure (Rauh et al., 2011). Working Memory (a component of IQ) is the ability to memorize new information, retain the memory short-term, and concentrate and manipulate information, all of which are considered predictors of the ability to learn and academic success (Whyatt et al., 2015). As assessed in by Rauh and colleagues (2011), Working Memory was not

confounded by lead (Pb) exposure and was not likely to be affected by socioeconomic or cultural conditions. Rauh et al. (2012) performed magnetic resonance imaging studies on 40 cohort children (5.9 – 11.2 years old) to see if CPF exposure in utero affected brain morphology. Brain cortical surface features were compared between children with high concentrations of CPF in cord blood plasma (n = 20; ≥ 4.39 pg/g) and those with lower concentrations (n = 20; < 4.39 pg/g). Numerous morphological differences were reported in the children in high CPF group, including enlarged superior temporal lobe, posterior middle temporal lobe, and inferior postcentral gyri bilaterally, as well as enlarged superior frontal gyrus, gyrus rectus, cuneus, and precuneus along the mesial wall of the right hemisphere. These children also showed frontal and parietal cortical thinning and an inverse dose–response relationship between CPF in cord blood and cortical thickness. Although expected, no sex differences in brain morphology were found between the high and low CPF groups (Rauh et al., 2012), but rather a reversal of sex differences in the high CPF group similar to those reported in animal models where early exposure reverses normal sex differences in learning, memory, and emotional behaviors (Hoberman, 1998; Levin et al., 2001; Aldridge et al., 2004; Aldridge et al., 2005a).

All cohort children not lost to follow-up (n=271) were assessed again at age 11 (range = 9.0 – 13.9)(Rauh et al., 2015). A total of 21 cohort children were diagnosed with a neurological, psychiatric, or learning disorder, the most common of which was ADHD. The children underwent a full battery of neurodevelopmental measures, including a test of motor function. CPF exposure was significantly associated with tremor in the dominant arm (p = 0.015), tremor in either arm (p = 0.028), and tremor in both arms (p = 0.027), and marginally associated with tremor in the non-dominant arm (p = 0.055) (Rauh et al., 2015). The authors state that morphologic changes appear to be related to lower IQs in these children and that the results support the notion that in utero exposure to CPF is associated with general cognitive deficits (Rauh et al., 2012) and potential central or peripheral nervous system effects later in life (Rauh et al., 2015).

II.K.5. Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) Cohort, Center for Children's Environmental Health Research, University of California, Berkeley

The CHAMACOS project within the UC Berkeley Center for Children's Environmental Health Research is a longitudinal birth cohort study of the effects of pesticides and other environmental exposures on the health of pregnant women and their children living in the Salinas Valley of California (Eskenazi et al., 2004). Eligible women were 18 or older and were less than 20 weeks pregnant at the time of enrollment (Oct 1999 – Oct 2000) through the Natividad Medical Center or one of five Clinicas de Salud de Valle de Salinas. The subjects were either farm laborers or were living with someone employed as a farm laborer in Salinas Valley, CA (Eskenazi et al., 2004).

Researchers evaluated nonspecific metabolites of OP pesticide exposure as well as specific metabolites for several pesticides, including CPF in urine at 13 weeks (mean) and 26 weeks (mean) of gestation. Levels of ChE in whole blood or BuChE in plasma in maternal and umbilical cord blood were measured in blood collected from mothers at 26 weeks of gestation and in the hospital before delivery (umbilical cord blood samples) (Eskenazi et al., 2004). A large proportion of women in the study had specific CPF metabolite values that were below the

limit of detection. For those samples in which TCPy was detected, the median value was 3.3 µg TCPy/L urine (range = 0.2 – 56.1 µg/L) (Eskenazi et al., 2004). No association was found between urinary concentrations of TCPy and any fetal growth outcome, although results indicated decreased gestational duration was associated with nonspecific urinary biomarkers of dimethyl OPs, such as malathion (Eskenazi et al., 2004). Results from questionnaires showed that very few home-use pesticides in the CHAMACOS study contained chlorpyrifos, and that the more likely sources of exposure included diet, indoor residues, or nearby agricultural use (Eskenazi et al., 2004).

Eskenazi and colleagues went on to explore multiple growth and development indices in the children of the CHAMACOS cohort, including the Bayley Scales of Infant Development for mental and psychomotor developmental indices at 6, 12, and 24 months of age. No association was found between decrements in any developmental indices and urinary concentrations of TCPy, a more specific marker of chlorpyrifos exposure. However, the nonspecific OP metabolite DEP in maternal urine was significantly associated with decrements in the child's mental development indices at 24 months, leading the authors to postulate that the observed association may be attributed to compounds other than just malathion or chlorpyrifos (Eskenazi et al., 2007). The investigation was expanded by considering the metabolic enzyme PON1 and its activity and genotypes/phenotypes in the cohort population, hypothesizing that there may be a subgroup of children that by virtue of their genetic makeup may be more susceptible to the adverse effects of OP exposure during pregnancy (Eskenazi et al., 2010). There were no statistically significant interactions between any nonspecific maternal urinary metabolites of OPs (DAPs) and enzyme measurements in relation to any of the neurobehavioral endpoints. There was a slightly stronger relationship of psychomotor development scores and maternal DAPs, particularly for the diethyl phosphate metabolites, among children with the lowest aryl esterase enzyme activity when compared to children with the highest PON1 activity (both measured in cord blood collected at the time of birth) (Eskenazi et al., 2010). There was a suggestion that children with $PON1_{-108T}$ allele showed a stronger association with general OP pesticide exposure in utero (as measured by prenatal DAPs) and the mental development indices, but the interaction was not significant (Eskenazi et al., 2010). Harley et al. (2011)went on to investigate infant PON1 genotype and activity. Infants with lower PON1 activity or those with a susceptible genotype ($PON1_{-108T}$) had a stronger association with shorter gestation duration and smaller head circumference at birth (Harley et al., 2011). Maternal metabolizing enzyme genotype and activity did not have the same association. The authors go on to postulate that PON1 may contribute to fetal growth impacts and decrements perhaps through an oxidative stress mechanism (Harley et al., 2011).

The children were followed up again at 3.5 and 5 years when both maternal and psychometrician assessments of behavior and neurodevelopment were conducted (Marks et al., 2010). The battery of tests conducted at each visit included visual attention, reaction time, accuracy, impulse control, motor activity, and distractibility. Prenatal DAPs were positively associated with attention problems and ADHD diagnoses. Composite measures of ADHD and attention were adversely related to both child urinary diethyl concentrations (reflecting recent OP exposure) and prenatal diethyl phosphate concentrations (Marks et al., 2010). Data for the more specific chlorpyrifos metabolite TCPy were not reported. Bouchard and colleagues (2011) went on to report that children 7 years old in the highest quintile of prenatal DAP concentrations have an average deficit of 7.0 IQ points compared to the lowest quintile of prenatal urinary DAP. Prenatal DAP concentrations were also associated with poorer scores for Working Memory,

Processing Speed, Verbal Comprehension, and Perceptual Reasoning (Bouchard et al., 2011). Child urinary DAP concentrations were not consistently association with any WISC finding, leading the authors to postulate that prenatal but not childhood DAP metabolites are associated with poorer intellectual development (Bouchard et al., 2011).

In 2016, Stein and colleagues published findings investigating early childhood adversities and the impact they may have on the association between prenatal OP pesticide exposures and the decrements in Full Scale IQ noted in the CHAMACOS cohort children. The authors collected information on potential sources of adversity in the homes of CHAMACOS cohort participants, including annual income, food insecurity, family structure, maternal depression, stressful life events, family conflict (including physical punishment), home learning environment, and social and emotional interactions between parent and child (Stein et al., 2016). Seventy percent (70%) reported income below the federal poverty line and 15% of mothers were at risk of clinical depression. Several types of adversity were significantly associated with decreased scores in Verbal Comprehension, Perceptual Reasoning, Working Memory, and Full Scale IQ. Adversity in relationships between parent and child were associated with decreases in Verbal Comprehension, Working Memory and Full Scale IQ (Stein et al., 2016). There were some sex differences in the outcomes, but overall there were stronger associations between prenatal OP exposures (as measured by nonspecific urinary metabolites) and IQ scores among children who are experiencing certain adversities (Stein et al., 2016).

II.K.6. Additional Studies and Pooled Analyses

Multiple studies continue to investigate associations between prenatal and early life exposures to OP pesticides and neurodevelopment in geographic locations as varied as Northern Ecuador, Cincinnati, Ohio, Norway, Brittany, France, Southeastern Spain, Mexico City, and Shenyang, China. A small sample of representative studies is summarized below.

In a prospective cohort of pesticide exposure in maternal and fetal biological matrices, 150 pregnant women scheduled for C-sections in New Brunswick, NJ from July 2003-2004 were recruited by convenience sampling (Barr et al., 2010). During the pre-operative procedures, 10 ml of maternal blood was collected. Within 15 minutes of delivery, 30-60 ml of cord blood was collected from the newborns. Both blood samples were analyzed for chlorpyrifos. CPF was detected in 98.5% of maternal samples and 62.8% of newborn sample, with many at or near the LOD. Maternal serum contained a mean level of 0.009 ng/g (SD = 0.87) and the cord blood contained an average of 0.55 ng/g (SD = 0.73). There were no associations with blood CPF levels are birthweight or birth length (Barr et al., 2010).

In a study of 119 children with ADHD ranging from 8 to 15 years old (a subset of NHANES subjects), researchers considered the association between urinary DAPs and ADHD subsets as defined in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). Children with higher urinary concentrations of DAPs, and especially dimethylthiophosphate (DMTP), were at higher risk of being diagnosed with hyperactive-impulse ADHD subtype (Bouchard et al., 2010). Metabolites from O,O-diethyl substituted OPs were not significantly associated with any increased risk of ADHD, whether defined strictly by the DSM-IV criteria or when including children taking ADHD medications. There were no significant sex- or age-related differences in the findings (Bouchard et al., 2010).

The Canadian Health Measures Survey (2007-9) considered biomarkers of exposure in 779 children 6-11 years old and their relation to growth and development (Oulhote and Bouchard, 2013). The children, who were representative of the general Canadian population, underwent blood and urine analysis, a household survey, and a Strengths & Difficulties Questionnaire (SDQ) to measure emotional symptoms, conduct problems, hyperactivity/inattention, peer problems, and pro-social behavior. Results indicated that total DAP levels decreased significantly with age (Oulhote and Bouchard, 2013). No significant association was found with any SDQ measurement of difficulty or any association with hyperactivity as found in Bouchard et al. (2010), even though total DAP levels in the Canadian children were higher than their American counterparts.

The Generation R cohort is a population based birth cohort in Rotterdam, The Netherlands. Over 8800 women enrolled during pregnancy and had delivery dates between April 2002 and January 2006. Eighty randomly selected women were recruited from the main cohort to provide 3 urine specimens throughout pregnancy and an additional 40 provided two urine samples during pregnancy (Spaan et al., 2015). All samples were tested for the non-specific DAP metabolites of OP pesticides. For all 6 DAP metabolites, the within-person variability exceeded the between-person variability, indicating poor-to-moderate reliability of one measurement as an indication of OP pesticide exposure throughout pregnancy. High total DEP metabolites were observed in women with a high daily vegetable, legume, and fruit intake (0.999, 1.001, and 1.002 nmol/g creatinine (lognormal-transformed, respectively).

Pooled analysis of 4 birth cohorts looked for association between metabolites in maternal urine and mental and psychomotor developmental indices (MDI and PDI, respectively), In Engel et al. (2016), the author notes that the geometric means for total DAP and total DMP concentrations were substantially higher in the CHAMACOS cohort than in the Columbia CCCEH, HOME (Health Outcomes and Measures of the Environment), and Mt. Sinai studies. There was significant heterogeneity in the associations between total DMP and total DAP and MDI, driven largely by a strong negative association from the CHAMACOS cohort. As such, the author states that this result argues against interpreting the pooled associations and that differences in the cohorts limited the interpretability of the overall pooled estimates (Engel et al., 2016). Different chlorpyrifos sources in the different cohorts also limit the ability to cross-compare results. For instance, subjects enrolled in the HOME study after the US EPA restriction on indoor use, so it is likely those subject may have received a higher proportion of their exposure through dietary means (and a higher quality diet high in fruits and vegetables) as compared with the two NY cohorts, whose subject enrollment spanned the period when indoor CPF restrictions were initiated (Engel et al., 2016).

Harley et al. (2016) considered fetal growth, exposure, and PON1 genotype and activity in the pooled data from the CHAMACOS, HOME, Mt. Sinai, and Columbia CCCEH cohorts. Total DEP concentrations measured in maternal urine during pregnancy in nmol/g creatinine were highest for the CHAMACOS cohort and lowest for the Columbia CCCEH cohort, with a pooled mean and standard deviation of 13.11 nmol/g creatinine (5.49).

Columbia CCCEH < HOME < Mt. Sinai < CHAMACOS

The authors found no significant associations between metabolites and birthweight, length, or head circumference in the pooled data of over 1000 pregnant women. However, there was a negative association between total DEP concentration and birthweight of the infants whose mothers exhibited the $PON1_{-108CC}$ genotype.

II.K.7. General Observations from Human Epidemiological Studies

As mentioned at the beginning of this section, CPF can be metabolized into dialkyl phosphate (DAP) metabolites. These metabolites are considered general metabolites of OP-containing compounds in the environment. Because each urinary metabolite has multiple sources, the presence of any DAP metabolite in urine (e.g., DMP, DEP, DMTP, DETP, etc.) may result from exposure to the parent compound (such as an OP pesticide) or an environmental degradate. DEP and DETP are common metabolites for many O,O-diethyl substituted pesticides such as diazinon, and therefore they cannot be considered specific markers of chlorpyrifos exposure. TCPy and DAP metabolites each represent one-half of the chlorpyrifos molecule and are produced in approximate equal-molar ratios (Barr and Angerer, 2006). Therefore, TCPy and DAP measurements should not be summed to determine CPF exposure, because in so doing, the exposure would be overestimated by a factor of 2 (Barr and Angerer, 2006).

Rather than the nonspecific urinary OP metabolites mentioned above, quantified chlorpyrifos levels in blood or blood product provides the best estimation of exposure to the parent pesticide. Chlorpyrifos exists in extremely low concentrations in blood compared to metabolites in urine (ppt versus ppb levels) (Barr and Angerer, 2006) and can be difficult to quantify above the analytical limit of detection. In addition, it requires obtaining a biological sample that is more difficult to collect than urine. Nevertheless, several epidemiological studies quantified chlorpyrifos in blood to characterize maternal and fetal exposure, most notably the Columbia CCCEH cohort (Perera et al., 2003; Whyatt et al., 2003; Perera et al., 2004; Whyatt et al., 2004). Over the course of cohort participant recruitment (c. 1998 – 2004), there were significant decreases in the level of parent compound measured in blood, indicating that changes in regulation have thus far resulted in significantly lower body burden of chlorpyrifos. In Whyatt et al. (2009), there was a significant decrease from 2001 – 2004 in urinary TCPy concentrations measured in participants. The percent of mothers with TCPy above the LOD steadily declined from 2001 (91%), to 2002 (84%), to 2003 (31%), to 2004 (29%). Both maternal and newborn blood samples had CPF levels below the LOD in all samples collected after 2002 (Whyatt et al., 2009).

II.L. The Toxicity ForeCaster (ToxCast™) Program

The Toxicity Forecaster (ToxCast^{TM}) program was launched by US EPA in 2007 as part of the Toxicity Testing in the 21st Century (Tox21) program in collaboration with the National Toxicology Program, the National Institutes of Health's National Center for Advancing Translational Sciences, and the Food and Drug Administration (http://www.epa.gov/chemical-research/toxicity-forecasting; accessed 12-2015). ToxCast was designed to prioritize chemicals based on the results of high-throughput screening assays indicating potential disruption of key biological pathwaysChemicals were selected for screening by US EPA (ToxCast and Tox21 collaborators), as well as international programs such as the Organization for Economic Co-operation and Development (OECD) and other stakeholder groups. The multi-phase ToxCast

program includes over 700 unique assays and 300 signaling pathways and to date has evaluated over 2000 chemicals with established or unknown toxicity, including cosmetics, drugs, pesticides, and environmental contaminants (Tice et al., 2013). ToxCast data may be used to elucidate biochemical mechanisms as well as common pathways for human disease outcomes. Ultimately, a goal of this US EPA program is to use the ToxCast hazard and exposure data predicted by computer modeling to facilitate chemical risk assessments and prioritization.

II.L.1. US EPA ToxCast Assays *In Vitro*

Results were obtained from the 11 ToxCast assay platforms that reported active results for CPF and CPF-oxon ("actives"): ACEA Biosciences, Inc. (ACEA), Apredica (APR), Attagene (ATG), Bioseek (BSK), CEETOX (Cyprotex), CellzDirect (CLD), Simmons Lab (NCCT), Novascreen (NVS) and Odyssey Thera (OT), the NIH Chemical Genomics Center (NCGC or Tox21) and zebrafish (National Health and Environmental Effects Research Lab - Padilla Lab [NEERL] or TANGUAY). The active results for CPF-oxon were included in the data presentation as none of the assay platforms have metabolic activation and it is known that CPF-oxon is the primary toxic metabolite of CPF. Table 17 provides detailed information on these assay platforms.

All assay results reported here were obtained from the Interactive Chemical Safety for Sustainability (iCSS) Dashboard (http://actor.epa.gov/dashboard/), the Endocrine Disruptor Screening Program Dashboard (http://actor.epa.gov/edsp21) and the FIFRA SAP Meeting on Integrated Endocrine Activity and Exposure-based Prioritization and Screening (http://www.regulations.gov/; Docket #: EPA-HQ-OPP-2014-0614). All assays reported on the dashboard were performed at multiple concentrations with the exception of Novascreen assays that were performed at one concentration only (25 µM all assays except 10 µM CYPs), and were reported on the iCSS Dashboard in the ToxCast Summary Files (http://www.epa.gov/ncct/toxcast/data.html).

Table 18. ToxCast Vendors and Assay Descriptions

Vendor	Organism Tissue	Cell Line Type	Biological Response	Target Family	Detection Technology
ACEA	Human Breast	T47D	Cell Proliferation	Cell Cycle	Label free
Apredica (APR)	Human Liver	HepG2	Mitochondrial depolarization	Cell morphology	Fluorescence
Attagene (ATG)	Human Liver	HepG2	Regulation of transcription factor activity	Background measurement	Fluorescence
Bioseek (BSK)	Human Tissues	Numerous primary cell types [a]	Regulation of gene expression	Depends on cell type system [b]	Fluorescence
CEETOX	Human Adrenal	H295R	Regulation of catalytic activity	Steroid Hormone	Spectrophotometry
CellzDirect (CLD/CRO)	Human Liver	Primary Cells	mRNA induction	Depends on assay design [c]	Chemiluminescence
Novascreen (NVS)	Human Proteins	Cell Free	Regulation of catalytic activity	Receptors, CYPs	Fluorescence
Simmons Lab (NCCT)	1. Rat Thyroid 2. Human Kidney	1.Cell Free 2. HEK293T	1. Regulation of catalytic activity 2. Cytotoxicity	1. Oxidoreductase 2. Cell cycle	1. Fluorescence 2. Luminescence

Vendor	Organism Tissue	Cell Line Type	Biological Response	Target Family	Detection Technology
NCGC (Tox21)	Human Kidney, Ovary, Breast	HEK293T	Regulation of transcription factor activity	Nuclear Receptor, cell morphology, DNA binding	Fluorescence, Reporter gene
Odyssey Thera (OT)	Human Kidney	HEK293T HeLa	Protein stabilization	Nuclear Receptor	Fluorescence
NHEERL or TANGUAY zebrafish	*Danio rerio* Whole animal [d]	NA	Malformations, neurobehavioral	Developmental Pathways	Visual/ Morphological

[a] Primary cultures from Primary human venule endothelial cells, Primary human vascular smooth muscle cells, Primary human dermal fibroblasts, Peripheral blood mononuclear + endothelial cells

[b] BSK tests for cytokine, cell adhesion, cell cycle, gpcr, growth factor, protease inhibitor, proteases depending on cell types assay.

[c] CLD tests for background measurement, CYP enzymes, transporters, transferase and lysase.

[d] Zebrafish assays are performed with chorion intact (Padilla et al., 2012) or with chorion removed (Tanguay et al., 2013; Truong et al., 2014). Zebrafish results are available with the other ToxCast results at: http://actor.epa.gov/dashboard/

II.L.2. ToxCast Assay Results for CPF and CPF-oxon

The results of ToxCast assays (reported as Concentration at 50% Activity: AC_{50}) that may be involved in CPF and CPF-oxon toxicity are shown in Table 17. Assay reactions are all without metabolic activation. However, a full complement of ToxCast assays was performed for both CPF and the major metabolite CPF-oxon (http://actor.epa.gov/dashboard/ accessed September 2017). All assay results and corresponding components or assay targets are compiled in histograms from the ToxCast Dashboard for CPF and CPF-oxon in Figure 7.

II.L.2.a ToxCast Assay Endpoints for Known CPF and CPF-Oxon Metabolism

CPF and CPF-oxon interaction with the following receptors or proteins is consistent with their metabolic pathway shown in Figure 3 above and described Table 18. Some of the assays are positive only with CPF-oxon because there is no metabolic activation to take CPF to the oxon form. Other assays may have high AC_{50} values because at high doses CPF becomes toxic and so the activity reported may or may not be due to a specific chemical/endpoint interaction.

- Human **AChE** and rat **BuChE** were active with CPF and CPF-oxon. The oxon form had greater sensitivity (lower AC_{50}) than CPF in NVS cell-free assays. CPF is associated with genes for AChE and BuChE (http://ctdbase.org/detail.go?type=chem&acc=D004390).

- **Cytochrome P450** (CYP) assays indicate that only CPF-oxon is active with the CYPS and the genes associated with CPF (**CYP1A1, CYP1A2, CYP3A4 and CYP2B6**) as would be predicted based on the metabolic pathway (Foxenberg et al., 2011). Aryl hydrocarbon hydroxylase receptor (**AhR),** also involved in xenobiotic oxidation, is active with both CPF and CPF-oxon (Fujita and Mannering, 1971). The oxon is more sensitive than CPF.

- **Farnesoid x receptor (FXR)** is an agonist and weak antagonist with CPF-oxon but is also active with CPF at higher concentrations. FXR is found in high levels in the liver and

intestines and interacts with peroxisome proliferators and retinoid x receptors (RXR) which also contribute to the metabolism of CPF (Jiao et al., 2015).

- **PXR (PXRE)** binds to the response element of the CYP3A4 promoter after forming a heterodimer with the 9-cis retinoic acid receptor (RXR), then regulating transcription of CYP3A4. Both CPF and CPF-oxon are active in the PXR assays. CPF is more sensitive then CPF-oxon (Kliewer et al., 2002).

- **Retinoid X receptor** (**RXR**) is activated by 9-cis retinoic acid and 9-cis-13,14-dihydro-retinoic acid and there are 3 main RXRs (RXRa, RXRb, RXRg). RXR hetero-dimerizes with constitutive androstenedione receptor (CAR), FXR, liver x receptor (LXR), peroxisome proliferator activated receptor (PPAR), pregnane x receptor (PXR), thyroid hormone receptor (TR), retinoic acid receptor (RAR), and vitamin D receptor (VDR). All of these genes interact with CPF, CPF-oxon, or both. RXR binding to agonist ligands results in promotion of downstream target gene mRNA production (Germain et al., 2006).

- **LXR** The **liver X receptor** (**LXRa or b**) is a transcription factors that is closely related to nuclear receptors such as the PPARs, FXR, and RXR. LXR regulates cholesterol, fatty acid, and glucose homeostasis and is classified as thyroid hormone receptor-like (NR1H3: LXRα; NR1H2: LXRβ). LXR hetero-dimerizes with 9-cis retinoic acid receptor (RXR) and, after activation, binds to LXR response element (LXRE). This receptor is activated by CPF (Song et al., 1994; Willy et al., 1995).

- **PPAR** is active with both CPF and CPF-oxon, but shows more sensitivity with CPF-oxon (detoxification) (Michalik et al., 2006).

- **CAR** interacts with PXR and functions as a sensor of endobiotic and xenobiotic substances. It activates metabolism of these compounds, functioning in conjunction with PXR to detoxify. CAR-regulated genes are members of the CYP2B, CYP2C, and CYP3A subfamilies, sulfotransferases, and glutathione-S-transferases. CPF-oxon is active with CAR nuclear receptor (Ueda et al., 2002; Wada et al., 2009).

II.L.2.b. Other ToxCast Assay Endpoints:

i. Central Nervous System (CNS):

CNS receptor assays show that CPF-oxon directly interacts with critical hormone regulating proteins in the brain. Notably these interactions have a high AC_{50} and are therefore not indicators of more sensitive pathways than the known AChE inhibition pathways (Table 18).

- The ɤ-aminobutyric acid receptor ($GABA_aR$) in the CNS (Hevers and Lüddens, 1998) is active with CPF in a cell-free assay.

- CPF-oxon also interacts with transmembrane G protein–coupled receptors (GPCRs) designed to detect compounds on the cellular exterior and activate internal responses (Wettschureck and Offermanns, 2005). Rat somatostatin inhibitory receptors are mediated by GPCR expressed in the anterior pituitary (NVS_GPCR_rSST). This interaction shows

the potential of CPF-oxon to affect growth hormone and other endocrine neurotransmitters in the brain. Although not a potent interaction, results from rat receptor assays nevertheless add to the potential for CPF-oxon to affect growth and development.

- Two rat opioid receptor assays are positive with CPF-oxon. Opioid receptors are also GPCR-coupled inhibitory proteins and are similar to the somatostatin receptors and function to affect pain (Janecka et al., 2004; Waldhoer et al., 2004; Reif et al., 2013). They are found primarily in the brain spinal cord and digestive tract.

- CPF-oxon interacts with the γ-hydroxybutyrate receptor in a brain tissue assay. This GPCR-coupled receptor normally binds γ-hydroxybutyric acid (GHB) a neurotransmitter as well as a psychoactive drug. Agonists and/or GHB receptor binding results in a stimulant effect mediated by an increased Na^+/K^+ current and increased release of dopamine and glutamate (Castelli, 2008; Castelli et al. 2003).

- CPF-oxon has activity with the glucocorticoid receptor (GR). This neuroendocrine receptor is part of the stress response regulated in the brain, including adaptation to stress, depression and other psychological states.

- CPF and CPR-oxon are both active in the vitamin D receptor element (VDRE) assay. Vitamin D is critical to brain and neurodevelopment both in utero and during childhood (Harms et al., 2011; Kočovská et al., 2012). Vitamin D deficiency has been associated with autism in children (Kočovská et al., 2012).

- Disruption of the RXR pathway, mentioned above, has been associated with neurodevelopmental effects, including pathways leading to schizophrenia (Goodman, 1998; Sun et al., 2010). This was one of the most sensitive assays with CPF.

Assays related to endocrine disruption show that CPF may interact with critical hormone systems (thyroid, androgen, estrogen), including inhibition of steroidogenesis even in the absence of metabolic activation. CPF interaction in receptors related to the steroidogenic, estrogenic, thyroid or androgenic pathways can directly affect human growth and development (Table 18). CPF is considered to be a weak estrogenic agonist on the EDSP dashboard (agonist Area Under the Curve, AUC = 0.0125; https://actor.epa.gov/edsp21/). CPF-oxon is a weak estrogen receptor antagonist with weak receptor binding. However the AC_{50}s for most of the endocrine-related effects are high (in the absence of metabolic activation) meaning that these are not likely to be primary targets and the positive results are likely non-specific interactions due to cytotoxicity.

Table 19. ToxCast Assays for Chlorpyrifos and Chlorpyrifos-oxon

Assay Name [a]	CPF AC_{50}	CPF Oxon AC_{50}
Acetylcholinesterase & Butyryl Cholinesterase Activity		
NVS_ENZ_rAChE	--	0.96
NVS_ENZ_hAChE	--	0.32
NVS_ENZ_hES (human plasma/BuChE ChE)	28.6	0.003
Cytochrome P450, Aryl hydrocarbon Hydroxylase & Aromatase Activities		
NVS_ADME_rCYP3A1	--	6.08
NVS_ADME_rCYP1A2	--	5.26
NVS_ADME_hCYP2C19	--	4.09
NVS_ADME_hCYP2C18	--	6.71

Assay Name [a]	CPF AC_{50}	CPF Oxon AC_{50}
NVS_ADME_hCYP2B6	--	9.04
NVS_ADME_hCYP1A2	--	3.8
NVS_ADME_hCYP1A1	--	8.49
CLD_CYP2B6_48hr	--	11.2
CLD_CYP1A2_48hr	--	4.1
CLD_CYP2B6_24hr	--	11.2
CLD_CYP1A2_24hr	--	0.404
CLD_CYP3A4_6hr	--	3.42
CLD_CYP2B6_6hr	--	11.9
CLD_CYP1A2_6hr	--	9.54
CLD_CYP1A1_6hr	--	5.84
TOX21_AhR_LUC_Agonist	41	--
ATG_Ahr_CIS_up	2.3	--
TOX21_Aromatase_Inhibition	--	14.4
Farnesoid x Receptor (NR1H4)		
TOX21_FXR_BLA_agonist_ratio	--	39.4
TOX21_FXR_BLA_antagonist_ratio	--	17
OT_FXR_FXRSRC1_1440	--	0.352
OT_FXR_FXRSRC1_0480	36.3	26.6
Retinoid x Receptor		
OT_NURR1_NURR1RXRa_0480	39.4	--
OT_NURR1_NURR1RXRa_1440	--	87.2
ATG_RXRb_TRANS_up	24.1	--
Pregnane x Receptor		
ATG_PXR_TRANS_up	4.3	--
ATG_PXRE_CIS_up	6.3	42.7
Liver x Receptor		
ATG_DR4_LXR_CIS_dn	35.2	--
Peroxisome Proliferator Activated Receptor		
TOX21_PPARg_BLA_antagonist_ratio	--	4.94
ATG_PPARg_TRANS_up	57.2	34
ATG_PPRE_CIS_up element	--	32.6
Constitutive Androstenedione Receptor		
NVS_NR_hCAR_Antagonist	--	21.9
Receptors in Human & Rat Brain		
NVS_GPCR_rSST rat forebrain; somatostatin receptor	--	13.4
NVS_GPCR_rOpiate_NonSelectiveNa	--	20.9
NVS_GPCR_rOpiate_NonSelective, forebrain opiate R	--	12
NVS_GPCR_rGHB forebrain, metabotropic glutamate R	--	21.8
NVS_LGIC_rGABAR_NonSelective	12.3	--
TOX21_GR_BLA_Antagonist_ratio	--	39.4
Vitamin D Metabolism		
ATG_VDRE_CIS_up	4.6	31.8
Thyroid Hormone		
TOX21_TR_LUC_GH3_Antagonist LXR PXR	79.7	35.8
Androgen Receptor		
OT_AR_ARSRC1_0960	85.1	--
TOX21_AR_BLA_Antagonist_ratio	--	40.7
Estrogen Receptor & Estrogen Metabolism		
TOX21_ERa_BLA_Agonist_ratio	--	1.55
TOX21_ERa_BLA_Antagonist_ratio	--	115
TOX21_ERa_LUC_BG1_Antagonist	--	43.7
OT_ER_ERaERa_0480	67	--
OT_ER_ERaERb_0480	64	--
OT_ER_ERbERb_0480	56.6	--
ATG_ERa_TRANS_up	20.2	33.8
ATG_ERE_CIS_up	34.3	--
Steroidogenesis		
CEETOX_H295R_11DCORT_dn	84.1	--
CEETOX_H295R_CORTISOL_dn	82.8	--
CEETOX_H295R_TESTO_dn	55.7	--

Assay Name [a]	CPF AC_{50}	CPF Oxon AC_{50}
CEETOX_H295R_ANDR_dn	54.8	--
CEETOX_H295R_PROG_up	39.8	--

[a] All assay abbreviations found at http://actor.epa.gov/dashboard/

Below is an illustration of CPF and CPF-oxon assays and their intended target families. There are more active assays in various target families for CPF-oxon versus CPF. This is expected since CPF-oxon is the active metabolite, while CPF requires metabolic activation which is not provided in the assays.

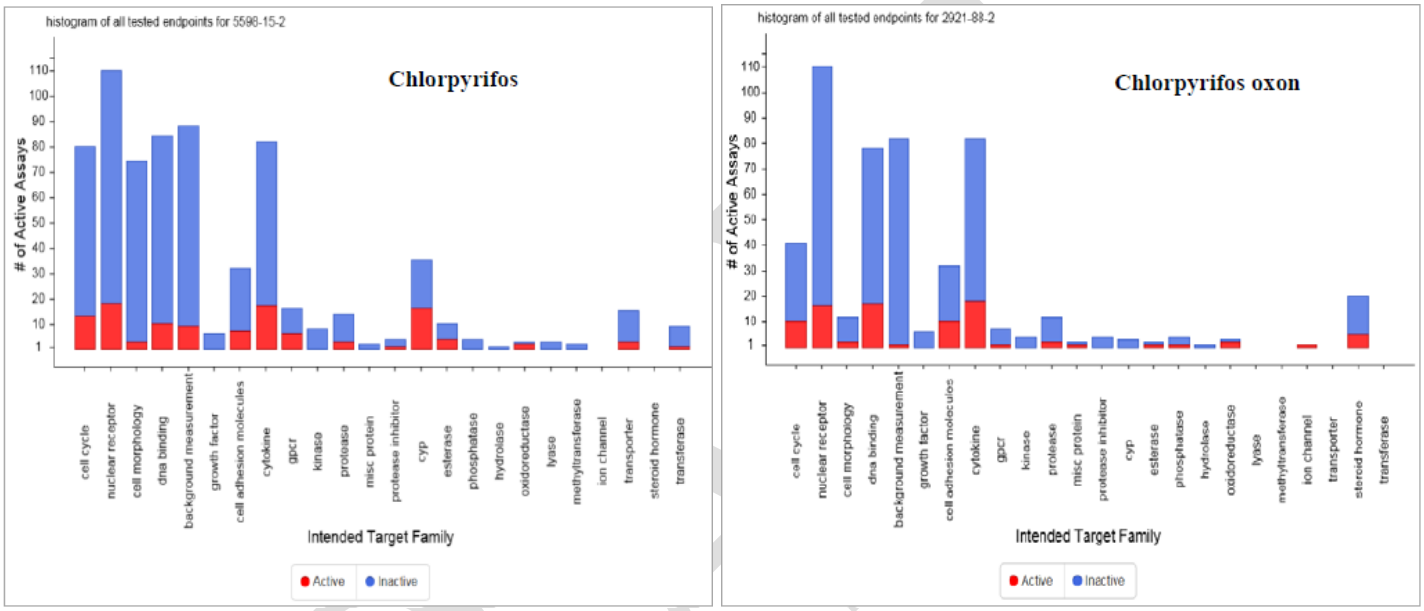


Figure 7. CPF ToxCast Assay Component Histograms

Active (red) and inactive (blue) ToxCast assays are shown for CPF and CPF-oxon, along with the respective intended target families

II.L.3. Toxicological Priority Index (ToxPi)

The Toxicological Priority Index (ToxPi) is a dimensionless index score calculated for each chemical as a weighted combination of all data sources that represents a formalized, rational integration of information from different domains. Visually is ToxPi represented as component slices each representing one piece (or related pieces) of information (Reif *et al.*, 2013; UNC, 2014). The ToxPi data in Figure 8 show relative ToxCast component activities between CPF and CPF-oxon. The input data were generated using AC_{50} values for all assays reported as active (ToxCast Dashboard: http://actor.epa.gov/dashboard/) and "100,000" for inactive assays. Inactives were included only in comparisons where at least one of the two compounds was active. The same scaling type ($-\log10^{(x)+6}$) was used for all ToxPi figures shown. The assay results were grouped into components specified on the ToxCast Dashboard as indicated in Figure 8 by color-coded slices. The unitless Toxicity Scores (Reif et al., 2010; Reif et al., 2013), calculated in the ToxPi program, were virtually identical (15.52 and 15.028 for CPF and CPF-oxon, respectively), despite the differences in the relative toxicities between components.

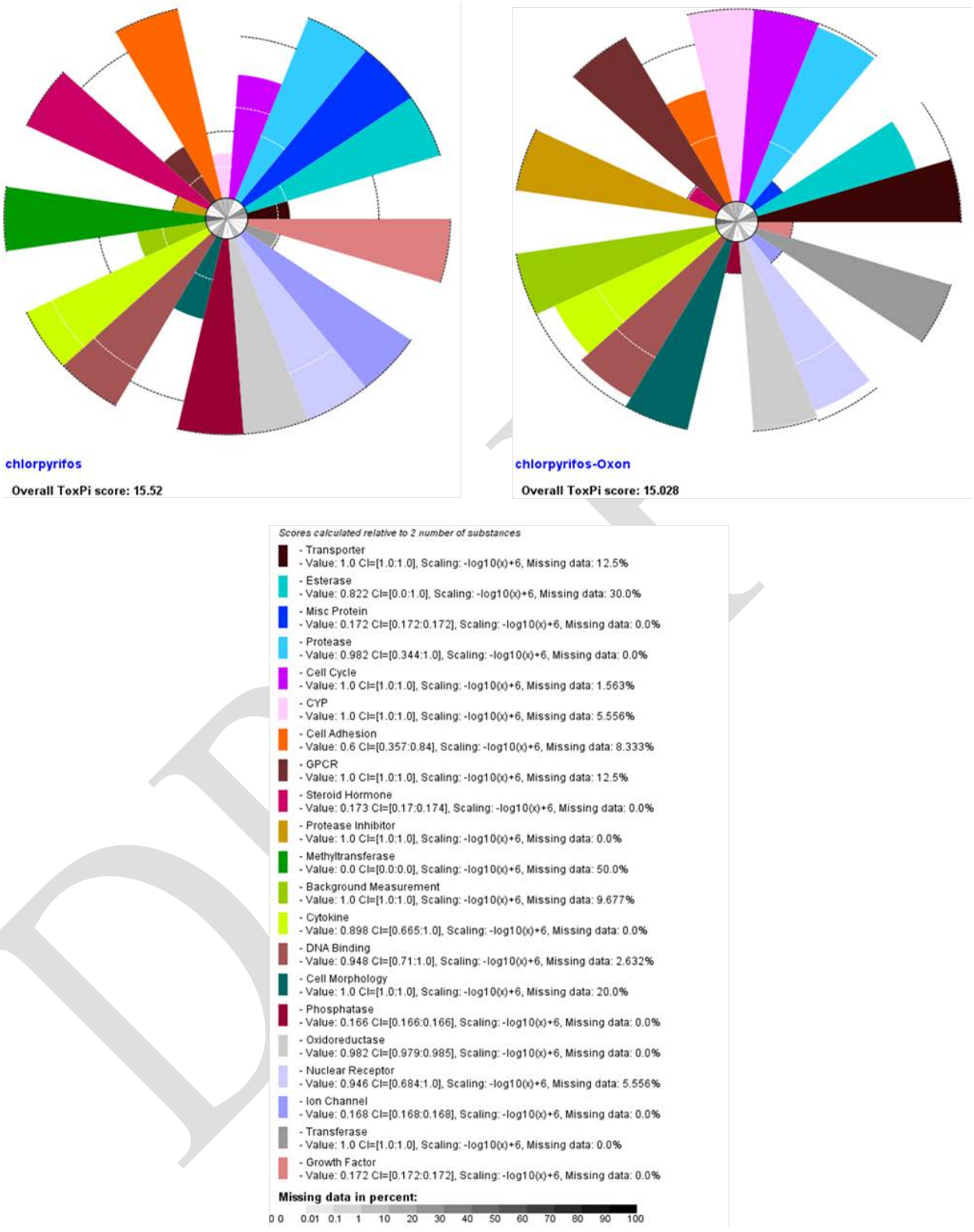


Figure 8. Toxicology Priority (ToxPi)

The ToxPi scale measured the presumptive components showing ToxCast assay activity for CPF (left) and CPF-oxon (right) (Data accessed: January 2017).

II.L.4. US EPA ToxCast Assays in Zebrafish

Zebrafish (zebrafish: *Danio rerio*) provide a model for studying effects of CPF *in vivo*. They share many developmental, anatomical, and physiological characteristics with mammals since molecular signaling is conserved across species (Padilla *et al.*, 2011; Sipes *et al.*, 2011; Padilla *et al.*, 2012; Tanguay, 2013; Tanguay *et al.*, 2013). They also require AChE for normal neurodevelopment (Behra *et al.*, 2002). For that reason, zebrafish are useful for studies of neurobehavioral developmental effects of AChE inhibitors like CPF.

DMSO was used as a vehicle in zebrafish studies. It is known to be neurotoxic at high concentrations (Kaisa et al. 2013; Maes et al. 2012) generating concern for augmented neurotoxicity when used as a vehicle in studies with CPF. In zebrafish DMSO must exceed 1.5-2%, depending on embryonic stage. At 2-4 cells and 4 hpf, 2.5% DMSO is non-toxic; at 1, 2 and 5 dpf, 2% DMSO is nontoxic and at 3 and 7 dpf 1.5% DMSO in solution is not toxic (Maes et al. 2006). Concentrations used in zebrafish studies are generally 0.01 – 0.64% (Hallare *et al.*, 2006; Maes *et al.*, 2012). The benefit of DMSO as a vehicle is to increase chemical uptake into the embryo order to aid in the elucidation of the mechanism of action.

Zebrafish embryos can reveal acute toxic effects of CPF since growth, development and behavior occur at such a rapid rate. Therefore, if a chemical is developmentally toxic in zebrafish, it would affect molecular pathways or processes that might be detected by phenotypic and/or neurobehavioral responses. These changes can then serve as indicators of affected pathways for target identification (Padilla *et al.*, 2011; Padilla *et al.*, 2012; Tanguay *et al.*, 2013; Truong *et al.*, 2014; Reif *et al.*, 2015). The two primary models consist of testing embryos with intact chorions (Padilla et al., 2012)or using embryos with the chorion removed (Tanguay *et al.*, 2013) (Results of each method on the ToxCast Dashboard: http://actor.epa.gov/dashboard/).

II.L.4.a. Zebrafish Method with Chorion Intact

Embryos (2 embryos/concentration/chemical) were exposed to each compound in a single treatment at 0.001 to 80 µM or a DMSO control (0.4% v/v). They were incubated in sealed plates within their aqueous media for ~4 days at 26±0.1 °C until hatching. They were then placed in an incubator and maintained on a 14:10 hour light:dark cycle. Each day through 120 hours (5 days) the animals had a complete change of medium with a fresh dose of compound. At 144 hours post-fertilization (hpf:6 days) each embryo/larva was evaluated for viability and developmental effects by use of a dissection microscope. The decision tree for collection of endpoints and descriptions of the categories and physical features within each category that were analyzed are presented in Padilla et al. (2011) and Padilla et al. (2012). Malformations received a "response" score for lethality and hatching status (Malformation Index [MI]: 20=non-hatching; 40=lethality; if alive and hatched, then MI = summation of aggregated scores across all categories of malformations for each condition) and the summation of all scores for all malformation categories was defined as the "Toxicity Score" (or "Terata Score"). In cases where larvae were alive and hatched then the Malformation Index and Toxicity score were equal. Graphically the Toxicity Score (y-axis) and chemical concentration (x-axis) were used in a custom "R implementation" (R Development Core Team, Vienna, 2011) of the Evolutionary Algorithm Dose Response Modeling (EADRM) (Beam and Motsinger-Reif, 2011) to determine a "hit" based on "efficacy," or response at the top asymptote of the sigmoidal fit (EMAX

Toxicity Score) (response): minimum cutoff is a score of 6.5 or one standard deviation above the mean of the vehicle control) and goodness-of-fit (R^2: minimum cutoff = 0.4). Chemical "potency" (AC_{50} and AC_{10} concentration at 10% maximal activity) and slope (W) were also determined (Figure 9).

Padilla et al. (2012) tested CPF-ethyl, which is the form of CPF evaluated in this risk assessment. The AC_{50} for CPF (8.5 µM; 2.97 µg/ml) was 21-fold greater than the AC_{50} for CPF-oxon (0.40 µM; 0.14 µg/ml). A Terata Score, or sum of all malformations and variations was reported for each chemical tested. CPF-oxon received the highest score (40) in the single 80µM test (CPF was not tested). Both compounds were tested up to concentrations producing a Terata Score of 40 in the concentration-response study (Figure 8). The slope was very steep for CPF between AC_{10} (3.0 µM) and the AC_{50} (8.5 µM). The AC_{10} in ToxCast assays is considered to be a NOEL equivalent (Judson et al. 2014).

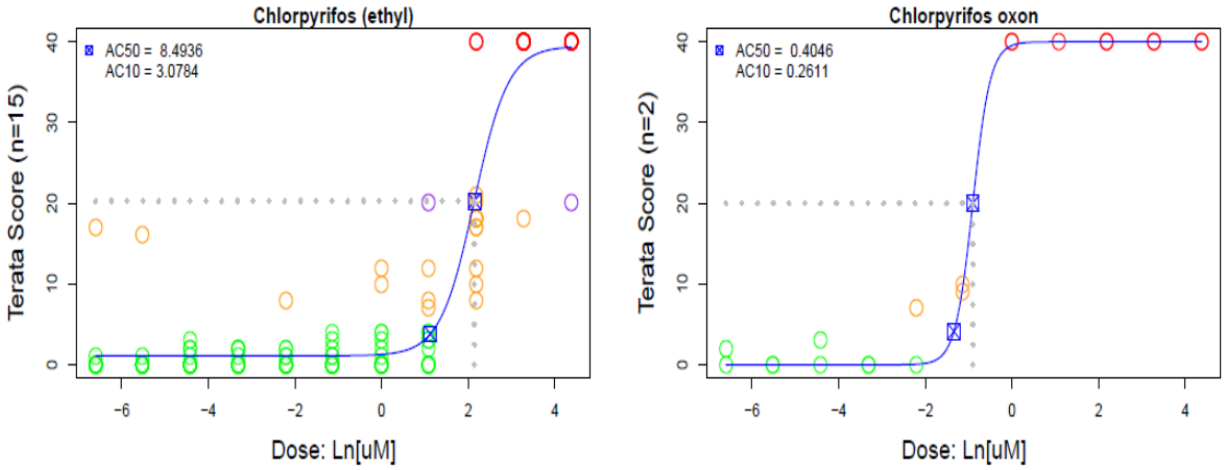


Figure 9. Terata Scores for CPF

Green = control levels; red = dead (Terata Score=40); purple= not hatched but alive (Terata Score ~ 20); yellow = animals alive and hatched (Terata score 8-20) (Padilla et al., 2012)

II.L.4.b. Zebrafish Method with Chorion Removed

Another method of treatment involved removal of the chorion from the zebrafish embryos prior to treating them with test compound in order to eliminate possible interference relating to absorption (i.e. exposure consistency), increase bioavailability, facilitate endpoint assessments and reduce confounders. Zebrafish (32/concentration) were treated with the test chemical at 0.064–640 µM (0.022 to 22 µg/ml: 10-fold serial dilutions) in DMSO (0.64% v/v). A positive control (5 µl trimethyltin chloride) was also used. Zebrafish were exposed daily with fresh media for 5 days (Truong et al., 2014). Plates were sealed to prevent evaporation and foil covered to reduce light exposure and kept in a 28°C incubator. Embryos were statically exposed (i.e., only one dose of test compound) until 120 hpf but at 24 hpf, they were assessed for photomotor response using a custom photomotor response analysis tool (PRAT) and for 4 developmental toxicity endpoints (MO24: mortality at 24 hpf, DP: developmental progression, SM: spontaneous movement, and NC: notochord distortion) (Truong et al., 2011). At 120 hpf, locomotor activity was measured using Viewpoint Zebralab (Saili et al., 2012; Truong et al., 2012) and assessed for 18 endpoints (Truong et al., 2011).

The graphs shown below indicated individual malformations by chemical (Figure12). Unlike what was observed with the Padilla method (i.e., chorion intact model) there were no effects for CPF. However, CPF-oxon showed mortality at 24 hours at all doses (MO24); developmental progress (inhibited) at 24 hours (DP24); mortality (mort); yolk sac (YSL), axis and trunk abnormalities were observed at ≥ 6.4 µM (2.24 µg/ml; pericardial edema (PE) and caudal fin (CF) abnormalities occurred at 64 µM (22.4 µg/ml). The increased mortality may have been due to the lack of a chorion barrier and a higher DMSO concentration (leading to higher permeability) than was used in the Padilla method.

The difference in toxic effects between the results of the chorionated versus dechorionated methods may be due to the different dosing methods as well as methods of scoring embryos or other unknown differences.

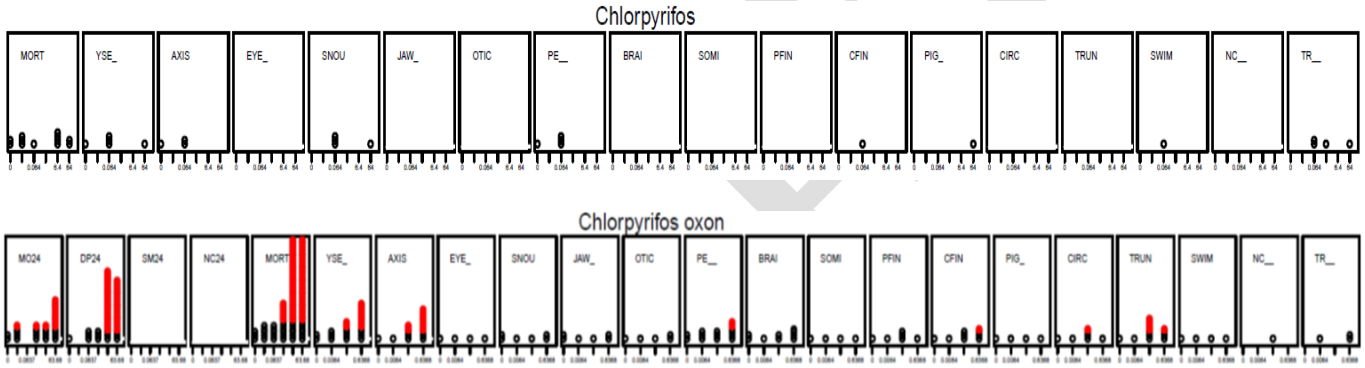


Figure 10. Morphological effects from CPF or CPF-oxon treatment in zebrafish

There were no effects for CPF.CPF-oxon caused mortality at 24 hours at all doses (MO24); developmental progress (inhibited) at 24 hours (DP24); mortality (mort); yolk sac (YSL), axis and trunk abnormalities were observed at ≥ 6.4 µM; pericardial edema (PE) and caudal fin (CF) abnormalities occurred at 64 µM (Truong et al., 2014).

Zebrafish behavioral effects were examined after treatment of embryos with CPF or CPF-oxon at doses of 0.0064 - 64 µM (daily: 5 days post-fertilization) (Reif et al., 2015). Animals were treated in the dark to which they adapted as they developed. At 24 hours hpf, animals received a light stimulus (30 second process) that was used to assess behavior as follows: Initial Phase (B): 1) a short prelight pulse (soft light background: "B"); Excitatory Phase (E): 2) immediately followed by a short pulse of bright light; 3) pause 9 seconds before the next light pulse; 4) a second pulse of light, and; Refractory Phase (R): 5) 10 seconds of dark . The animals were videotaped during the process and their behavior was later analyzed. Results showed that CPF only showed significant effects during the excitatory phase but not during B or R (these were within the control range). CPF-oxon showed effects from B at 6.4 µM, E at 0.64 µM, and no effects during R (within control range). This means that CPF-oxon caused noticeable behavioral effects at a 10-fold lower dose 0.64 µM when exposed to the bright light pulse as opposed to the background light. This is also the dose at which other developmental effects were observed as shown in (Figure 10) (Truong et al., 2014). CPF showed behavioral effects only for the bright pulse of light and only at the highest dose (64 µM); however CPF showed no morphological developmental effects at any dose (Figure 10).

II.L.4.d. Zebrafish Results From Laboratories Not Related to ToxCast (Chorion Intact)

Levin et al. (2003) used CPF at 0.028 µM and 0.28 µM (0.01 and 0.10 µg/ml: 0.02% DMSO vehicle) on zebrafish embryos (chorion intact) for 5 days. Animals were tested for behavioral effects intermittently up to 26 weeks. Mortality was high at 0.28 µM (0.10 µg/ml: 5/12 died) at 38 weeks (0/13 DMSO; 1/16 at 0.028 µM [0.01 µg/ml]). At 0.028 µM (0.01µg/ml), zebrafish had effects on average choice accuracy, decreased spatial discrimination, increases in average latency response when the animals were first tested (20 weeks). This indicated that neurobehavioral/ learning/cognition effects occurring after treatment with CPF in an embryonic stage were not reversible. Levin et al. (2004) then treated zebrafish for effects of CPF on swimming behavior. Tested at day 6, animals showed decreased swimming activity and decreased habituation of swimming activity at 0.28 µM (0.10 µg/ml). These effects involve the central nervous system (CNS: >0.028 µM [0.01 µg/ml]) as well as peripheral nervous system (PNS: 0.28 µM [0.10 µg/ml]: muscular).

Zebrafish embryos (chorion intact) were treated with 0.28 µM (0.10 µg/ml) CPF for various periods (0–1, 0–2, 0–3, 0–4, 0–5 days post-fertilization [dpf]) to optimize exposure for learning and memory impairments (Sledge et al., 2011). Persistent effects from dpf 5 to adult included: decline in brain dopamine and norepinephrine levels, decreased habituation to startle, "trend toward increased overall startle response," (Sledge et al., 2011) page 742) decreased escape diving response, increased swimming activity and lower learning rate. When placed in a new environment (novel tank exploration test) the zebrafish also showed a decrease in escape diving response and increased swimming after 5 days of treatment when tested at 3 months.

Jin et al. (2015) evaluated neurobehavioral and teratogenic effects in zebrafish (chorion intact) after CPF treatment at 0 (DMSO), 0.028, 0.084, 0.28, 0.84 µM (0.010, 0.030, 0.10 and 0.3 µg/ml) for 48, 60 or 96 hours post fertilization. Results at 96 hpf showed neurobehavioral (↓swim distance) effects related to stimulation of light/dark photoperiod transition at 0.084 µM and teratogenic effects (spinal deformities, pericardial edema) at 0.84 µM zebrafish. Neurobehavioral effects occurring after treatment with CPF in an embryonic stage were not reversible. In addition, AChE inhibition was increased at 0.28 µM and AChE mRNA was decreased at 0.84 µM, oxidative stress-related enzyme levels (↓GSH, ↓GST, ↑catalase, MDA, SOD) were affected at ≥0.028 µM and the transcriptional levels of genes related to neurotoxicity were affected at ≥0.028 µM.

CPF was shown to affect anxiety-related behaviors in zebrafish (chorion intact) at ≥0.01µM (0.0028 µg/ml) when they were exposed for 7 dpf (Richendrfer et al., 2012a). The altered behaviors exhibited included decreased swim speed and thigmotaxis (edge preference). There was a decrease in fish on the edge of the dish both with and without visual stimuli (decreased anxiety) at ≥0.01 µM. At 1.0 µM fish showed tails that curled up and the fish twitched but could not swim. They also had shorter body at 1.0 µM. There were no effects on avoidance behavior.

At 0.001 µM (0.00028 µg/ml) CPF, there were no changes in swim speed, thigmotaxis, or avoidance behavior and at 1 µM (0.028 µg/ml) CPF there were both behavioral and teratology effects. Thigmotaxis is an anxiety-related behavior in zebrafish larvae (Richendrfer et al., 2012b) and this behavior alteration appears to be directly related to exposure to low doses of CPF especially 3-5 dpf.

Zebrafish embryos (chorion intact) were exposed to CPF at 0, 0.28, 0.71, 1.42, 2.14 and 2.85 µM for 48 hours (media change every 12 hrs; 10 embryos/dose in triplicate) to assess the potential for endocrine disruption (Yu *et al.*, 2015). CPF was shown to increase hatching time in a dose-related manner. Indicators of cell proliferation and cell apoptosis were affected based on mRNA expression of c-myc, cyclin D1, Bax and Bcl-2, which are closely related to cell proliferation and cell at 48 h. Apoptosis occurred at 2.31 and 2.85 µM, indicating that endocrine disruption could be occurring. Increases in vitellogenin (VTG), a protein is a biomarker for vertebrate exposure to environmental estrogens, was assessed in the zebrafish embryos. The mRNA expression of VTG was increased at ≥0.71 µM but the estrogen receptor alpha data were equivocal. It appears that as with the ToxCast results, endocrine disruption occurs at doses higher than those affecting behavior and AChE inhibition.

Zebrafish (Tübingen strain) embryos were treated with 0 (0.01% acetone v/v) or 0.71 µM (0.25 mg/L) CPF at 2 hours post fertilization (hpf) for 24 hours (Liu et al. 2015). This CPF dose was tested and shown not to increase mortality or malformations compared to adult animals. Embryo media was changed at 12 hours. The acetone vehicle was shown not to affect protein expression (Hallare *et al.*, 2006). At 24 hours the major organ systems, somites, pronephros, heart and central nervous system have developed. The zebrafish proteome was mapped to indicate the effects on stress-related proteins. Results showed that many proteins involved in zebrafish development were affected including 9 that are related to CPF detoxification (heat shock protein, aldehyde dehydrogenase 2, and glutathione S-transferase M), cytoskeleton structure, protein translation, signal transduction and lipoprotein metabolism. Three of the up-regulated proteins were associated with detoxification (aldehyde dehydrogenase 2 (ALDH2) precursor, glutathione S-transferase M) and stress response (shock protein (Hsp60)).indicating a protective response in the zebrafish embryos exposed to CPF. Six down-regulated proteins were associated with cytoskeleton structure (Type I cytokeratin, enveloping layer), protein translation, signal transduction and lipoprotein metabolism. Detoxification-related proteins were presumably induced in response to CPF exposure (protective) while down-regulation of Apo lipoprotein A (a major protein component of HDL particles in plasma) may lead to disruption of the oxidative stress response.

II.L.4.d. Zebrafish and Acetylcholinesterase Inhibition (Intact Chorion)

AChE activity is critical to zebrafish nervous system development as has been demonstrated by Behra et al. (2002). They developed a genetically altered zebrafish strain (*ache: chorion intact*) which totally eliminated AChE activity (ACh hydrolysis) in homozygotes. The embryos with the mutant phenotype (-/-*ache*) have defective innervation (PNS) and muscle fiber development resulting in premature death of sensory neurons (Behra et al., 2002). Initially embryos are motile but when primary sensory neurons die, the lack of innervation of muscle fibers results in paralysis. "The neuromuscular phenotype in *ache* mutants is suppressed by a homozygous loss-of-function allele of the α-subunit of the nicotinic acetylcholine receptor (nAChR), indicating that the impairment of neuromuscular development is mediated by activation of nAChR in the mutant" (Behra et al., 2002).

Yen et al. (2011) examined the possibility that the CPF MOA also involves inhibition of zebrafish AChE resulting in hyperstimulation at cholinergic synapses and subsequent loss of neuromuscular activity by neuronal death. They examined AChE inhibition in zebrafish embryos

(intact chorion) after exposure to 0.28 µM (~0.105 µg/ml) throughout a 5 day post-fertilization (dpf) treatment. AChE was inhibited at 2 dpf and steadily increased until it peaked at 80% inhibition at 5 dpf when compared to DMSO control. Subsequently zebrafish movements were tracked at 6 dpf (one day after 0-5 dpf exposure). At 0.28 µM CPF exposures reduced locomotor activity by 35% 0.28 µM CPF (~0.105 µg/ml). This exposure level was about the same as used by Jin et al. (2015) and Levin et al. (2004) where neuromuscular effects were also observed.

A study by Richendrfer and Creton (2015) examined AChE inhibition and neurobehavioral toxicity in zebrafish (chorion intact) treated at lower doses of CPF (0.001, 0.01, 0.1 µM or ~0.00028, 0.0028, 0.028 µg/ml) during various treatment windows (1-5 dpf or late development 3-5 dpf). As shown by Jin et al. (2015), 80% of AChE is inhibited at 0.28 µM (0.105 µg/ml). This study was meant to examine what effects occurred at even lower doses. Results showed that AChE was significantly decreased only at 0.1 µM (0.035 µg/ml) CPF, whereas at ≥0.01 µM (0.0028 µg/ml) CPF there was a significant increase in abnormal behavioral ("fish at rest" was increased; swim speed was decreased after 1-5 dpf treatment). Zebrafish treated during 3-5 dpf showed a significant decrease in fish with a preference for being on the side or on the edge of their swim lane (signifies decreased anxiety), a decrease in swim speed and an increase in "fish at rest" at ≥0.01 µM (0.0028 µg/ml) with a complete absence of AChE inhibition. These results show that at CPF concentrations 10-fold lower than those that inhibit AChE can affect the behavior of zebrafish during development. A summary of the zebrafish studies is below in Table 19 (Oliver *et al.*, 2016).

Table 20. Summary of Zebrafish Studies

Study design (exposure, conc., solvent)	DMSO	Chorion +/-	ENDPOINT	Ref[a]
Conc: solvent control, 0.028, 0.084, 0.28, 0.84 µM (0.01, 0.030, 0.10 and 0.3 µg/ml; N = 30-50 eggs per assay x 4 reps; Exposure: 48, 60 or 96 hrs post fertilization; CPF purity provided	Yes, conc. Not stated	No mention assume +	Hatchability: ↓ ≥ 0.084 µM at 60 hpf; no effect at 96 hr Heart rate: ↓ at ≥ 0.084 µM at 48 hrs Body length: ↓ at ≥0.01 µM 96 hrs At 96 hpf: Locomotion (distance & speed): ↓ at ≥0.084 µM; ↓AChE activity 0.28 µM (≥ 100 ppb); ↓mRNA & proteins levels at 0.84 µM; ↑ oxidative stress-related enzyme levels (↓GSH, ↓GST, ↑catalase, MDA, SOD), ↑transcriptional levels of genes related to neurotoxicity, & immunotox at ≥0.028 µM	1
Conc.: solvent control, 0.028, 0.28 µM (0.01, 0.10 µg/ml); Exposure: 5 days (120 hrs); + recovery phases with behavioral testing (20-38 weeks) Analytical confirmation: No	0.2 µl/ml; 0.02%*	No mention assume +	Survival: ↓ at 0.28 µM at 26 &32 weeks, but not 20 or 38 weeks. Choice accuracy & spatial discrimination: ↓ ≥0.028 µM at 10 and 100 ppb (dose responsive); Response to stimuli: slowed responses at 0.010 µM and quickened response time at 0.1 µM (1-6 & 7-12 sessions)	2
Conc: solvent controls; CPF 80 µM single dose; or 0.001, 0.004, 0.012, 0.03, 0.11, 0.32, 1, 2.96, 8.8, 26.6 & 80 µM (dose-response); N= 4 embryos/conc. (single dose); 2 embryos per conc (dose-response) Exposure: 5 days; CPF-ethyl; Analytical confirmation: No; AC_{50} = Toxicity score (they assigned descriptive data a numerical score: 40=lethality; 20=nonhatching, larva alive & hatched Toxicity Score =MI	0.4% (v/v)*	+	CPF ethyl & CPF-oxon; Single conc. CPF-ethyl only: Toxicity score: 40 (lethal) at 80 µM; AC_{50}: 0.4046 µM; CPF--oxon (8 replicate sets); AC_{50}: 8.4936 µM. CPF-ethyl CPF slope between AC_{10} (3.0 µM; 1.05 µg/ml) & AC_{50} (8.5 µM; 2.97 µg/ml). Embryo death with CPF occurred at about 20 µM and with CPF-oxon the animals were killed at about 1 µM (20:1 toxicity ratio).	3
Conc.: solvent control, 0.001, 0.01, 0.1, 1 µM 7 days post fertilization; Analytical confirmation: No	0.1%*	-	↓Edge preference with and without visual stimuli (decreased anxiety) at ≥0.01 µM; with visual stimuli ≥0.1 µM; 1.0 µM fish showed tails that curled up and showed twitching but could not swim; 1.0 µM shorter body length, lethargic.	4

Study design (exposure, conc., solvent)	DMSO	Chorion +/-	ENDPOINT	Ref[a]
Conc.: solvent control, 0.001, 0.01, 0.1 µM; Exposure: 1-5 dpf or late development 3-5 dpf Analytical confirmation: No	0.1% *	+	Swim speed: ↓0.1 & 0.01 µM; ↑effects during the 3-5 dpf window. AChE activity significantly ↓only at 0.1 µM (0.035 µg/ml); ↑ abnormal behavioral (↑"fish at rest"; swim speed ↓after 1-5 dpf treatment) at ≥0.01 µM; during 3-5 dpf ↓fish with a preference for being on the side or on the edge of their swim lane (signifies decreased anxiety) at ≥0.01 µM with a complete absence of AChE inhibition.	5
Conc.: 0.0064–64 µM CPF, CPF-oxon, CPF-methyl; no mention of solvent control; Exposure: 120 hpf N = 16 per plate x 2 plates	0.64% *	-	CPF: no dose dependent trends in any of the 18 markers (morphology & locomotor activity); CPF-oxon: ↑ mortality, yolk sac edema; body axis effects with dose dependent trends. CPF-methyl: no dose-dependent trends apparent; mortality, eye, snout, jaw, truncated body, touch response effects ↑ at 64 µM	6
Conc.: solvent control; 0.003 - 1 µM CPF & CPF-oxon Exposure: 24 to 48 or 72 hours	0.1%*	+	CPF: No significant effect on AChE activity at 48 or 72 hrs; Uptake after exposure to 1 µM was 11.06, 32.48, & 36.86 ng/embryo, respectively. CPF-oxon: dose-dependent ↓ in AChE activity at 48 & 72 hours; sign. ↓ 0.03 - 1 µM; Morphology: ↑ pericardia edema, body axis curvature & ↓pigmentation at 1 µM only. Swim behavior: ↓ at ≥ 0.1 µM (dose-dependent trend); Axonal growth in sensory neurons: ↓at 1 µM (were recoverable)	7
Conc.: solvent control, CPF 0.3 - 30 µM; Exposure: 5 days N=10 (survival), 30 (AChE & motility)	0.1%*	No mention assume +	↑ mortality at ≥ 3 µM; 80%↓AChE activity at 0.28 µM 5 dpf; 35% ↓Locomotor activity at 0.28 µM	8
Conc.: solvent control, CPF 0.71 µM; Exposure 24 hr., N=150/dose; protein mapping for stress & developmental effects	0.1% acetone	No mention assume +	Mapping of up-regulating detoxification (aldehyde dehydrogenase 2 (ALDH2) precursor, glutathione S-transferase M) & stress response (shock protein (Hsp60)) proteins; 6 down-regulated proteins for cytoskeleton structure (Type I cytokeratin, enveloping layer), protein translation, signal transduction & lipoprotein (Apo-A) metabolism	9
Conc: solvent control, CPF 0, 0.28, 0.71, 1.42, 2.14 and 2.85 µM for 48 hours (media change every 12 hrs)	0.1% Acetone	+	↑apoptosis at >2.14 µM, mRNA effects on cell proliferation indicators at all doses (mRNA expression of c-myc, cyclin D1, Bax and Bcl-2); ↑ VTG at ≥0.71 µM	10

References: 1. Jin et al. 2015; 2. Levin et al. 2003; 3. Padilla et al. (2012); 4. Richendrfer et al., 2012a; 5. Richendrfer & Creton, 2015; 6. Truong et al. 2014; 7. Yang et al. 2011; 8. Yen et al. 2011; 9. Liu et al. 2006; 10. Yu et al. 2015

Abbreviations: DMSO=Dimethyl sulfoxide; hfp: hours post-fertilization; dpf: days post-fertilization

Table adapted from Oliver *et al.* (2016).

III. HAZARD IDENTIFICATION

Pesticide risk assessment starts with hazard identification (hazard ID) in which toxic endpoints are identified from studies performed usually in accordance with US EPA's Health Effects Test Guidelines (US EPA, 2000b) or from the open literature. Once the toxic endpoints are identified, a No-Observed-Effect-Level (NOEL), a Benchmark Dose Lower Estimate (BMDL), or Point of Departure (PoD) is obtained. This is the highest dose at which no biologically or statistically significant adverse effect for the primary exposure route (oral/dermal/inhalation) is expected to occur relative to the control group. The hazard ID for CPF focused on 10% RBC AChE inhibition as well as neurodevelopmental and neurobehavioral toxicity in humans.

Note that in our selection of critical studies, we do not include mammalian studies where DMSO was used as a vehicle or where chlorpyrifos exposure was by a subcutaneous route. DMSO is not acceptable as an oral vehicle since it may exacerbate neurotoxic effects (Carr and Nail, 2008) and subcutaneous administration is not an applicable route of human exposure for CPF.

III.A. Acute (1 dose) and Short-Term (~2 weeks) Toxicity

The profile of acute CPF toxicity has been extensively described (Eaton *et al.*, 2008; Testai *et al.*, 2010; Koshlukova and Reed, 2014; US EPA, 2014a). The database for the acute toxicity for CPF consists of Health Effects Test Guideline studies submitted to DPR by registrants as well as open literature studies that were considered by HHA scientists to be relevant and well-performed. Acute exposure to toxic levels of CPF results in the typical signs and symptoms of cholinergic toxicity: salivation, lacrimation, urination and defecation (Eisler, 2007).

III.A.1. Acute and Short-Term Oral Toxicity

The overt effects from acute or short-term oral exposure to CPF in adult rats, mice, and rabbits include cholinergic reduced body weight and food intake, enlarged adrenals, and increased resorptions. Fetal and pup overt toxicity in these species include increased post-implantation loss, reduced live fetuses, reduced survival, reduced body weights, reduced crown-rump length, increased delayed ossification, reduced pup growth, delayed pinna unfolding, preputial separation (M), vaginal patency, delayed vaginal opening, reduced brain size, reduced motor activity, reduced auditory startle habituation and latency to response, and reduced neuromotor function. The NOELs for these overt effects were at doses higher than those for AChE inhibition.

Carr et al. (2013)and Carr et al. (2014)were the only studies reporting overt toxicity with the same NOEL as for AChE inhibition (Table 7 and Table 13). Overt effects involved inhibition of endocannabinoid enzymes in the central nervous system. The studies explored effects of CPF on two serine hydrolase enzymes which are involved in endocannabinoid degradation, including monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH). The associated neuromodulatory lipid endocannabinoids were 2-arachidonoylglycerol (2-AG), which was metabolized by MAGL, and anandamide (AEA) which was metabolized by FAAH. These cannabinoids are essential in neurodevelopment, but their levels in CNS are controlled by MAGL and FAAH to keep ligand concentrations at optimal levels (Anavi-Goffer and Mulder, 2009). Results showed that FAAH was inhibited to a greater extent and for a longer duration than brain AChE in rat pups. Supporting these findings are studies by Carr et al. (2015a); Carr et al. (2015b); Mohammed et al. (2015) which showed significant neurobehavioral effects in rat pups treated with the same regimen at 0.5 mg/kg/d. Therefore, FAAH inhibition may be a more sensitive endpoint than AChE inhibition for neurodevelopment. However, sufficient information is not yet available about this system to use it for establishing a critical NOEL. Instead, these effects will be evaluated in relation to database uncertainties for potential increased sensitivity in infants and children.

The acute oral NOELs (or PoDs) used by US EPA were obtained from their PBPK-PD model based on 10% RBC AChE inhibition data from human studies (Nolan et al., 1984; Kisicki et al., 1999; Smith et al., 2011; Smith et al., 2014). Although the animal model provided a lower NOEL than the PBPK-PD model, it is preferable to use human data from well-conducted studies when

available. The chlorpyrifos PBPK-PD model has been thoroughly evaluated and critiqued by several sources, including publication of the model in peer-reviewed journals (Gearhart et al., 1990; Timchalk et al., 2002a; Timchalk et al., 2002b; Timchalk et al., 2006; Lowe et al., 2009; Hinderliter et al., 2011; Smith et al., 2011; Poet, 2013; Poet et al., 2014; Smith et al., 2014). It has also been reviewed by the SAP (US EPA/SAP, 2010; US EPA/SAP, 2012; US EPA/SAP, 2016)and US EPA (2014a). Because the PBPK-PD model is based on human data obtained through dosing studies and metabolic factors derived from human tissues, US EPA has designated an interspecies uncertainty factor (UF) = 1x (US EPA, 2014b), which HHA also used. Therefore, the PoDs for acute oral CPF exposures are as follows:

PoD for infants < 1 year old = 0.60 mg/kg/d
PoD for young children ages 1-2 years = 0.581 mg/kg/d
PoD for children aged 6-12 years = 0.53 mg/kg/d
PoD for youth aged 13-19 years old = 0.475mg/kg/d
PoD for females of childbearing age (13-49 years old) = 0.457 mg/kg/d

The lowest acute oral PoD was for females of childbearing age (13-49 years old) (0.457 mg/kg/d), and will be used for dietary exposure assessments (see Table 21 below).

For acute oral spray drift risk characterization, the steady-state PoD for children ages 1-2 years old was used (0.099 mg/kg/d). It is appropriate to use steady-state for California exposure scenarios in which crops are treated for a few hours every 10 days because AChE inhibition is slowly reversed over approximately 26 days. At 10 days, acetylcholinesterase inhibition is still 50% in plasma and approximately 20% in RBCs, resulting in accumulated inhibition in those exposed for the duration of the season of treatment (Nolan et al., 1984).

III.A.2. Acute Dermal Toxicity

Acute dermal CPF toxicity from a single administration was assessed in adult rats (M/F) and a decrease in plasma and RBC AChE was observed (Calhoun and Johnson, 1988). Multiple studies showed no AChE inhibition in human plasma ChE after a single treatment at a single dose (5.0 mg/kg/d) (Nolan *et al.*, 1982; Hoberman, 1998; Mattsson *et al.*, 1998; Maurissen *et al.*, 2000; Marty and Andrus, 2010; US EPA, 2011b). No overt effects were reported. The NOELs were 1.0 and $\geq$ 5.0 mg/kg/d for rats and humans, respectively. The rat dermal study performed by Chen et al. (1999)had the lowest NOEL of 1.0 mg/kg/d based on plasma and RBC AChE inhibition at the LOEL (10 mg/kg/d). This study was not performed according to US EPA Health Effects Test Guidelines. In addition, the toxicological significance of plasma and RBC AChE inhibition by itself is uncertain, especially in animals compared to humans. Therefore, HHA used the PBPK-PD-generated steady-state dermal PoD of 11.89 mg/kg/d for females of childbearing age and 134 mg/kg/d for children aged 1-2 years old to evaluate the acute spray drift dermal exposure scenarios.

III.A.3. Acute Inhalation Toxicity

Male and female rats were treated with CPF in an aerosol (nose only) in a single exposure and showed plasma, RBC and lung AChE inhibition (Hotchkiss et al., 2010). The LOEL was 3.7 mg/m^3 (1.0 mg/kg/d) based on ChE inhibition in plasma, RBC and lung at every dose. In another

study, female rats administered CPF as a vapor (to saturation) showed no effects on plasma, RBC, and brain AChE at the only dose tested via nose only (17.7 ppb/0.254 mg/m^3) (Hotchkiss et al., 2013). The study of greatest interest for risk assessment is the one performed with aerosols, since that is the most likely medium for human inhalation exposure in California as shown in this document. Poet and colleagues (2015)incorporated an inhalation exposure route into the PBPK-PD model. Inhalation parameters used in the model were from the aerosol study in rat by Hotchkiss et al. (2010). The PBPK-PD model provided good comparisons for the critical metabolic parameters (e.g., plasma chlorpyrifos, oxon, and TCPy concentrations; ChE in plasma, RBC and brain). In vivo rat data were then used to validate the PBPK-PD model. Poet (2015)indicated that the PBPK/PD predictions for aerosol (particulate) inhalation exposure with respect to CPF, CPF-oxon, and TCPy in plasma as well as ChE in plasma, RBC, and brain was validated with data from the rat acute CPF aerosol inhalation study (Hotchkiss *et al.*, 2013; Poet, 2015). US EPA did not anticipate acute inhalation exposure for their residential scenarios. They instead generated PoDs for steady-state inhalation exposure for two critical subpopulations, children aged 1-2 years-old (PoD = 2.37 mg/m3) and females of childbearing age (PoD = 6.15 mg/m3) (US EPA, 2014a).

III.B. Subchronic Toxicity

Subchronic CPF toxicity was described and reported in the US EPA 2007 RED, the 2011 US EPA Preliminary Human Health Risk Assessment, and the 2014 US EPA Revised Human Health Risk Assessments (US EPA, 2007; US EPA, 2011a; US EPA, 2014a), and in the HHA Summary of Toxicology Data (Appendix 1). Summaries of registrant-submitted studies used in consideration for developing the subchronic endpoints are listed in Table 19, below. All studies are considered acceptable according to US EPA Health Effects Test Guidelines except the supplemental (non-Guideline) 6-week dietary CPF study performed in Beagle Dogs (Marable et al., 2001) designed to evaluate clinical signs, metabolism, and/or AChE inhibition.

III.B.1. Subchronic Oral Toxicity

Overt subchronic effects from CPF treatment included reduced body weights and feed consumption, increased clinical signs, neurobehavioral effects in FOB and motor activity, changes in urinalysis, hematology, and clinical chemistry values, changes in organ weights, increased adrenal zona fasciculata fatty vacuolization and altered adrenal tinctorial properties in adults, and reduced pup weights and pup survival. However, the most sensitive endpoint from the five dietary and one gavage studies shown below is AChE inhibition. In some cases a NOEL was not observed. A $BMDL_{10}$ of 0.03 mg/kg/d was calculated by US EPA (2011b) based on a weight-of-evidence from 5 multidose studies performed in rats(Hoberman, 1998; Mattsson *et al.*, 1998; Maurissen *et al.*, 2000; Marty and Andrus, 2010; US EPA, 2011b).

US EPA calculated an oral steady-state (21-day) PoD of 0.078 mg/kg/d from the PBPK-PD model. As mentioned earlier, because the PBPK-PD model is based on human data obtained through dosing studies and metabolic factors derived from human tissues, US EPA has designated an interspecies uncertainty factor (UF) = 1x (US EPA, 2014a), which HHA also used. Therefore, the PoDs for steady-state oral CPF exposures are as follows:

PoD for infants < 1 year old = 0.103 mg/kg/d
PoD for young children ages 1-2 years = 0.099 mg/kg/d
PoD for children aged 6-12 years = 0.090 mg/kg/d
PoD for youth aged 13-19 years old = 0.080 mg/kg/d
PoD for females of childbearing age (13-49 years old) = 0.078 mg/kg/d

The lowest steady-state oral PoD (0.078 mg/kg/d for females of childbearing age) will be used for subchronic/chronic dietary. The oral steady-state PoD for children 1-2 yrs old (0.099 mg/kg/d) was used to assess acute spray drift risk.

III.B.2. Subchronic Dermal Toxicity

No NOEL was achieved after 5 mg/kg/d CPF dermal treatment in rats (the only dose tested) (Calhoun and Johnson, 1988)(Table 19). Nor was a NOEL achieved in another CPF dermal study performed in mice (Krishnan et al., 2012), although a LOEL was established at 101 mg/kg/d based on reduced plasma ChE in adults and pups. Therefore, animal data for subchronic dermal exposure was not available for critical NOEL selection. The PBPK-PD model used by US EPA predicted steady-state 10% RBC AChE inhibition based on TCPy as a biomarker for CPF exposure in humans (Poet et al., 2003; Timchalk et al., 2007; Timchalk and Poet, 2008; Lowe et al., 2009; Smith et al., 2011; Smith et al., 2014). The modeled steady-state dermal PoDs are therefore useful to HHA for risk characterization since an animal NOEL is not available and because the PBPK-PD model is well described for the relevant subpopulations at risk. Females aged 13-49 years old (ss PoD = 23.6 mg/kg/d) and children ages 1-2 years old (ss PoD = 134 mg/kg/d) were used as the critical NOELs to assess dermal steady-state spray-drift risk. These PoDs were selected as the critical NOELs to evaluate subchronic spray drift inhalation exposure to CPF (see Table 19).

III.B.3. Subchronic Inhalation Toxicity

A 13-week study in rats established a NOEL of 0.010 ppm(0.143 mg/m^3) based on decreased AChE activity (Newton, 1988). It is important to note that the study was performed with CPF vapor and not aerosol. US EPA reported PoDs for steady-state (subchronic 21-day) inhalation exposure for two critical subpopulations: children 1-2 years-old (PoD = 2.37 mg/m^3) and females 13-49 years-old (PoD = 6.15 mg/m^3) (US EPA, 2014a). These PoDs were selected as the critical NOELs to evaluate subchronic spray drift inhalation exposure to CPF (see Table 21). As discussed earlier, the inhalation steady-state PoDs for females of childbearing age and children 1-2 years old were used to assess acute spray drift risk.

Table 21. Subchronic AChE and Overt Effects of Chlorpyrifos and the Respective NOELs and LOELs

Species	Exposure Duration	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[a]
Oral					
Rat F-344 M/F	Diet 28 d	↓ plasma ChE ↓body weights, body weight gains, feed consumption; ↑clinical signs & urinalysis, hematology, clinical chemistry & organ weight effects; ↑fatty vacuolization of the adrenal zona fasciculata	**Overt 1.0** **Plasma ChE 0.05**	Overt 5.0 AChE 0.1	1*

Species	Exposure Duration	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[a]
Rat SD M/F	Diet 2-Gen Repro	Parental:↑ vacuolation in zona fasciculate, altered tinctorial properties in this tissue; ↓ plasma and RBC AChE Pup: ↓pup weights & pup survival	**Overt Parental/Pup: 1.0** **ChE: 0.1**	Overt Parental/Pup: 5.0 AChE: 1.0	2*
Rat F-344 M/F	Diet 13 wk Neurotoxicity	↓ plasma and RBC AChE ↑ clinical signs, ↑FOB, motor activity effects	**Overt: 1.0** **ChE: 0.1**	Overt: 5.0 AChE: 1.0	3*
Rat Long-Evans F	Gavage c.o. 4 wk	↓ plasma, RBC and brain ChE ↑miosis & clinical signs; motor slowing and/or ↓ motivation (↑actual total delay, ↑ void trials, ↓#'s nose-pokes/trial).	Overt: 1.0 ChE: --	Overt: 3.0 AChE: 1.0	4*
Rat SD M/F	Gavage c.o. GD 6-20	↓RBC, Plasma & Brain ChE	**ChE $BMDL_{10}$: 0.03**	BMD_{10}[f] 0.06	7
Beagle Dog M/F	Diet 6 wk	↓RBC AChE	ChE: --	AChE: 0.5	6
Dermal					
Rat F-344 M/F	21d, 6hr/d, 5d/wk	No effects	--	No LOEL > 5.0	8
Mice Balb/c M Adult (150 d) Pup (18 d)	4 hr/d, 2 weeks: 1 dose level administered on the tail	Pup/Adult: ↓ plasma ChE	Pup/Adult: --	Pup/Adult: 101	9
Inhalation					
Rat CD(SD): Crl M/F	Vapor, Nose-only; 6 hr/d, 5d/wk 2 wks	No RBC, plasma, or brain ChE inhibition	--	LOEL >12 ppb	10
Rat F-344 M/F	Vapor, Nose-only; 6 hr/d, 5d/wk, 13 weeks	No RBC, plasma, or brain ChE inhibition	--	LOEL>20.6 ppb (0.295 mg/m^3)	11
Rat -344 M/F	Aerosol, Nose-only; 6 hr/d, 5 d/wk, 13 wk	↓Plasma ChE	10 ppb (0.143 mg/m^3)	20 ppb (0.286 mg/m^3)	12

[a] References: 1. Szabo et al. (1988); 2. Breslin et al. (1991); 3. Shankar et al. (1993); 4. Maurissen et al. (1996); 5. Boverhof et al. (2010); 6. Marable et al. (2001); 7. Mattsson *et al.* (1998); Maurissen *et al.* (2000); Marty and Andrus (2010); US EPA (2011b) 8. Calhoun and Johnson (1988); 9. Krishnan et al. (2012); 10. Landry et al. (1986); 11. Corley et al. (1986); 12. Newton (1988). *The study was acceptable to HHA based on FIFRA guidelines.

III.C. Chronic Toxicity

Chronic CPF toxicity was described and reported in the US EPA RED and Revised Human Health Risk Assessments (US EPA, 2007; US EPA, 2011a; US EPA, 2014a) and in the HHA Summary of Toxicology Data (Appendix 1). Registrant-submitted studies under consideration for the chronic endpoints are summarized in Table 21. All are considered acceptable according to US EPA Health Effects Test Guidelines (US EPA, 2000b).

III.C.1. Chronic Oral Toxicity

Chronic studies available for CPF endpoint determination show that the most sensitive endpoint in rats (Young and Grandjean, 1988; Crown, 1990; US EPA, 2000b), mice (Gur, 1992), and Beagle dogs (McCollister et al., 1971) was ChE inhibition (Table 10 and Table 11). An

BMD_{10}/$BMDL_{10}$ for RBC AChE inhibition was estimated for pregnant female rats ($BMDL_{10}$ = 0.03 mg/kg/d) by US EPA in their 2011 Preliminary Human Health Risk Assessment (US EPA, 2011a) based on data from Hoberman (1998), Mattsson et al. (1998), Maurissen et al. (2000)and Marty and Andrus (2010)Marty and Andrus (2010).

Overt chronic effects from CPF treatment included reduced body weight, reduced food and water consumption, yellow perineal stain, and increased clinical signs such as hepatocytic fatty centrolobular vacuolation, ulcerative dermatitis, panophthalmitis or endophthalmitis keratitis, accumulation of alveolar macrophages in lungs and septal thickening, cystic bulbourethral gland, vacuolation of the adrenal zona fasciculate, diffuse retinal degeneration/atrophy, and cataracts (Young and Grandjean, 1988; Crown, 1990)(Crown 1990; Young and Grandjean 1988a). The NOELs for these overt effects were at doses higher than those for AChE inhibition.

The PBPK-PD steady-state PoDs described earlier was also applied to chronic exposure (Table 11). Although steady-state values are higher than the $BMDL_{10}$ (estimated at 0.03 mg/kg/d), they are based on human data in a well-vetted model. Since RBC AChE reaches steady-state within 2-3 weeks, the use of a steady-state value for a chronic PoD can be rationalized (US EPA, 2014a). HHA used same steady-state PoDs described for subchronic oral toxicity here to describe chronic oral CPF exposures:

PoD for infants < 1 year old = 0.103 mg/kg/d
PoD for young children ages 1-2 years = 0.099 mg/kg/d
PoD for children aged 6-12 years = 0.090 mg/kg/d
PoD for youth aged 13-19 years old = 0.080 mg/kg/d
PoD for females of childbearing age (13-49 years old) = 0.078 mg/kg/d

The lowest steady-state oral PoD (0.078 mg/kg/d) for females 13-49 years old will be used for subchronic/chronic dietary characterization. Steady-state for oral PoDs for children (1-2 yrs old) was used for spray drift exposure assessments.

III.C.2. Chronic Dermal Toxicity

There were no chronic dermal toxicity studies available for CPF (Table 20). The US EPA PBPK-PD model estimated PoDs for steady-state dermal exposure (21-day) for several critical subpopulations (children 1-2 years-old: 0.13425 mg/kg/d; children 6-11 years-old: 0.02575 mg/kg/d; youths 11-16 years-old: 0.01395 mg/kg/d; females 13-49 years-old: 0.0236 mg/kg/d [highest dermal exposure]) (US EPA, 2014a). Since CPF RBC AChE inhibition reaches a steady-state within a 21 d period, HHA selected PoDs from children 1-2 years old and females 13-49 yrs-old (134.25 mg/kg/d and 23.6 mg/kg/d, respectively) to evaluate chronic dermal exposure to CPF spray drift.

III.C.3. Chronic Inhalation Toxicity

There were also no chronic inhalation toxicity studies available for CPF (Table 20). US EPA (2014a) reported a 10% RBC AChE inhibition PoD for steady-state (subchronic 21-day) inhalation exposure based on the PBPK-PD model for two critical subpopulations (children 1-2 years-old: 2.37 mg/m^3; females 13-49 years-old: 6.15 mg/m^3). Steady-state for ChE inhibition is

achieved within 21 days. Therefore, the steady-state modeled PoDs were selected by HHA to evaluate chronic inhalation exposure from CPF spray drift (Table 22).

Table 22. Chronic AChE and Overt Effects of CPF and the Respective NOELs and LOELs

Species	Exposure Duration	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref [a]
Oral					
Rat F-344 M/F	Diet 2 yr	↓ plasma ChE; ↓body weight; perineal yellow; vacuolation of the adrenal zona fasciculate; ↑diffuse retinal degeneration	Overt: 1.0 ChE: 0.05	Overt: 10 ChE: 0.1	1*
Rat F-344M/F	Diet 2 yr	↓ plasma, RBC & brain ChE; ↓body weight; diffuse retinal atrophy & cataracts	Overt: 1.25 ChE: 0.01	Overt: 50 ChE: 0.1	2*
Rat SD F	Gavage c.o. GD 6-20 (DNT)	↓ RBC and brain ChE	ChE BMDL10: 0.03	ChE BMD10: 0.06	3*
Mouse CD-1	Diet 79 wks	↓ plasma, RBC and brain ChE; ↓body weight & food & water consumption; ↑clinical signs; ↑Hepatocytic fatty vacuolation: centrilobular, Ulcerative dermatitis; Keratitis, panophthalmitis or endophthalmitis; accumulation of alveolar macrophages in lungs & septal thickening; bulbourethral gland cystic dilatation	Overt: 0.78 ChE: <0.078	Overt: 7.9 ChE: 0.078	4*
Dog Beagle M/F	Diet 2 yr	↓ plasma (0.03), RBC (1.0) and brain AChE (0.03): only ChE tested, no overt effects.	Overt: >3.0 ChE: 0.03	Overt: 3.0 ChE: 0.1	3*

[a] No chronic dermal or inhalation studies.

[b] References: 1. Young and Grandjean (1988); 2. Crown (1990); 3. McCollister *et al.* (1971); US EPA (2011a); 4. Gur (1992); 7. Hoberman (1998); Mattsson *et al.* (1998); Maurissen *et al.* (2000); Marty and Andrus (2010); US EPA (2011b). *The study was acceptable to HHA based on FIFRA guidelines

III.D. Summary of Critical NOELs Used for HHA Risk Assessment

Table 23 summarizes the critical NOELs and endpoints selected for evaluating oral, dermal, and inhalation exposure from diet and spray drift. The PBPK-PD model is advantageous for risk assessment because 1) the uncertainties and lack of NOELs for various animal studies make it difficult to use their data for PoD estimation; 2) the PBPK-PD model has been peer reviewed and published in the open literature; and, 3) the PBPK-PD model can be adjusted based on the subpopulation exposed and the duration of exposure in a standardized manner (e.g., the model incorporates acute oral, steady-state oral, dermal, and inhalation exposure parameters designed to simulate human exposure scenarios for given age or gender groups expected to result in 10% RBC AChE inhibition) (US EPA, 2014a). As such, the PBPK-PD modeled values from US EPA 2014 Revised Human Health Risk Assessment were used for HHA's dietary and drinking water MOE calculations primarily for females (13-49 yrs old) and children (1-2 yrs old). Note that steady state values were used for acute oral, dermal, and inhalation bystander spray drift exposure.

Table 23. Summary of Critical NOELs for All Exposure Durations

Exposure Route[a]	PBPK-PD PoDs (US EPA, 2014a)							
	Infants < 1 yr old		Children 1-2 yrs old		Children 6-12 yrs old		Females 13-49 yrs old	
	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]
Dietary (food only) and Drinking Water Exposures								
Drinking H_2O (oxon ppb)	1,183	217	3,004	548	7,700	1,358	5,285	932
Food (mg/kg/d)	0.600	0.103	0.581	0.099	0.530	0.090	0.467	0.078
Non-Dietary Exposures								
Incidental Oral (mg/kg/d)	--	--	--	0.101	--	--	--	--
Dermal (mg/kg/d)	--	--	--	134.25	--	--	--	23.60
Inhalation (mg/m^3)	--	--	--	2.37	--	--	--	6.15

a-PoDs are human equivalent doses derived from PBPK/PD model based on 10% inhibition of the RBC AChE activity after an acute (single day, 24 hr) or steady-state (21-day) exposure to CPF in humans (US EPA, 2014a).PoD from parent compound CPF was used for all exposure routes except for drinking water where PoD from CPF-oxon was used.
b- This assessment used SS oral (non-dietary), dermal, and inhalation PoDs to estimate the risk from spray drift and aggregate exposures.
c- Acute PoDs for CPF-oxon in ppb (µg/L) were converted into internal doses (mg/kg/d) using default drinking water consumption and body weight values
d- Steady-state dermal PoDs were developed assuming exposure duration of 1.5 hours per day for 21 days (US EPA, 2014a).
e- Steady-state inhalation PoDs were developed assuming exposure duration of 1 hour per day for 21 days (US EPA, 2014a).

IV. EXPOSURE ASSESSMENT

IV.A. Exposure Assessment of Non-Occupational Bystanders

IV.A.1. Introduction

The purpose of this exposure assessment is to evaluate non-occupational bystanders' exposure to CPF due to off-site movement (i.e., spray drift) of the product from agricultural applications in California. Other exposure scenarios will be addressed in an addendum, if needed. In California, field applications of CPF are made by both aerial and ground-based methods, and the latter includes ground boom and airblast (Dawson et al., 2012). For agricultural applications, 24 products with the aerial and (or) ground-based application methods are currently registered in California; their formulations include aqueous concentrate, emulsifiable concentrate, and wettable power (Table 24). In this exposure assessment, granular products are omitted because the focus is on spray drift following application of a liquid.

Table 24. CPF Products Labeled for Use in the Production of an Agricultural Commodity in California

Product Name	EPA Registration No.	Formulation
Bolton Insecticide	279-3581-AA	Emulsifiable Concentrate
Bolton Insecticide	67760-112-AA	Aqueous Concentrate
Chlorpyrifos 4E Ag	66222-19-AA	Emulsifiable Concentrate
Cobalt	62719-575-AA	Emulsifiable Concentrate
Cobalt Advanced	62719-615-AA	Emulsifiable Concentrate
CPF 4E	83222-20-AA	Emulsifiable Concentrate
Drexel Chlorpyrifos 4E-Ag	19713-520-AA	Emulsifiable Concentrate
Drexel Lambdafos Insecticide	19713-671-AA	Emulsifiable Concentrate

Product Name	EPA Registration No.	Formulation
Dursban 50W	62719-72-ZA	Wettable Powder
Eraser	62719-220-AA-71058	Emulsifiable Concentrate
Govern 4E Insecticide	62719-220-AA-55467	Emulsifiable Concentrate
Hatchet	62719-220-ZC	Emulsifiable Concentrate
Lock-On Insecticide	62719-79-ZA	Emulsifiable Concentrate
Lorsban Advanced	62719-591-AA	Aqueous Concentrate
Lorsban-4E	62719-220-ZA	Emulsifiable Concentrate
Nufos 4E	67760-28-AA	Emulsifiable Concentrate
Quali-Pro Chlorpyrifos 4E	66222-19-ZA	Emulsifiable Concentrate
Stallion Brand Insecticide	279-9545-ZA	Emulsifiable Concentrate
Stallion Insecticide	279-9545-AA	Emulsifiable Concentrate
Vulcan	66222-233-AA	Emulsifiable Concentrate
Warhawk	34704-857-AA	Aqueous Concentrate
Warhawk Clearform	34704-1077-AA	Emulsifiable Concentrate
Whirlwind	62719-220-AA-5905	Emulsifiable Concentrate
Yuma 4E	62719-220-ZA-1381	Emulsifiable Concentrate

IV.A.2. Exposure Scenarios Development

IV.A.2.a. Exposure Duration

Based on the number of applications allowed and the application intervals for high-use crops on the CPF product labels, short-term exposure is determined to be the focus of this bystander exposure assessment due to spray drift. DPR defines short-term exposure as lasting seven days or less (Andrews, 2001). The rationale for this determination is presented below.

For aerial applications, crops predominantly involved are alfalfa, cotton, corn (forage/fodder), and sugar-beets. Alfalfa is the crop with the most frequent repeated applications allowed, a total of 4 per season by some labels (e.g., Lorsban Advanced [62719-591-AA]) and Bolton Insecticide [67760-112-AA]]. Other labels allow 4 applications per year, with a single application allowed per cutting (e.g., Nufos 4E [67760-68-AA]). The minimum interval between applications is 10 days. The University of California (UC) Cost and Return Study for Alfalfa grown in Sacramento County assumes an average cutting of 7 times per year: "April, May, June, July (twice), August, and September" (Long et al., 2015). This suggests that with the exception of July, the shortest interval anticipated between applications is about a month. Even in July, the applications are probably spaced far enough apart to consider bystanders exposed to a series of acute exposures. Corn, cotton, and sugar-beets are each allowed 3 applications per season, with a minimum interval of 10 days.

For airblast applications, crops predominantly involved are tree fruits, nuts, and grapes. Foliar applications to citrus are limited to twice per year. Minimum application intervals are 30 days. Foliar applications to tree nuts are limited to 3 times per season. Minimum application intervals are 10 days. Grapes are only permitted one application per season with no potential of repeated exposure. For groundboom applications, the predominant crop is broccoli. According to the UC Cost and Return study for broccoli, there are normally 2 crops per year (Dara et al., 2012). This suggests that there could be as many as 6 applications to a field per year, and the minimum application interval is 10 days.

Based on the analysis above, exposure to CPF due to off-site product movement is considered to be a series of short-term exposures. For a given crop treatment, the exposure interval is no more frequent than 10 days.

IV.A.2.b. Spray Drift Exposure Assessment Approach

For assessing the short-term exposure due to off-site movement of CPF, this exposure assessment adopted the method of US EPA (Dawson et al., 2012): spray drift modeling coupled with the 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, 2017). For the post-application assessment, US EPA standard operating procedures (SOP) for residential exposure assessment were followed (US EPA, 2013).

Technical description of these models has been detailed elsewhere (Teske et al., 2002a; Teske et al., 2002b; Barry, 2017). Both AgDRIFT and AGDISP models were used to estimate off-site horizontal deposition of CPF at different distances downwind: 1000 feet for the aerial and 300 feet for ground boom and airblast applications. Table 25 shows the application types and model parameter values for use in estimating the drift deposition. These scenarios and parameter values were chosen to represent the reasonable worst case application conditions so that spray drift is not underestimated for the application scenarios assessed. To ensure horizontal deposition estimates are consistent with the application methods of airblast and ground boom in California, the number of swaths modeled was 40 for airblast and 60 for ground boom instead of the AgDRIFT default of 20 swaths. AGDISP was used to estimate horizontal deposition and 1 hour time-weighted average air concentrations (mg/m^3) of CPF at vertical heights of 1.7 ft and 5 ft. The vertical heights of 1.7 ft and 5 ft represent the breathing zones of children 1-2 years old and females 13-49 years old, respectively. The aerial application exposure scenarios evaluated in this exposure assessment used the estimated air concentrations for each specific scenario. For airblast and ground boom, the AGDISP model was used to produce surrogate air concentrations using a default aerial application (AT802A 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. Similar to the deposition estimates, these time-weighted air concentrations are the reasonable worst case air concentrations based on the parameters listed in Table 25.

Table 25. Application Type Scenarios for Chlorpyrifos Deposition Estimates

Application Type	Sub-Type	Parameter Value	Nozzle Droplet	No. of Swaths[b] (Coverage)[c]
Aerial	Fixed-Wing (AT802A)	10 mph wind; 20% RH; 90°F[a]	Medium	50 (206.6)
	Rotor-Wing (Bell 205)	10 mph wind; 20% RH; 90°F[a]	Medium	50 (190.4)
Ground Boom	Low Boom (20 inches above the canopy)	regression equation	M-to-C	40 (37.2)
	High Boom (50	regression equation	M-to-C	40 (37.2)

	inches above the canopy)			
Orchard Airblast	Sparse/Young	regression equation	NS	60 (7.05)
	Dormant Apple	regression equation	NS	60 (7.05)

Abbreviations: M-to-C, medium to coarse; NS, not specified; RH, relative humidity
[a] Meteorological conditions contributed to the highest drift deposition (i.e., worst case condition).
[b] Number of swaths to cover the field sizes in California.
[c] Equivalent square acreage covered by the total number of swaths.
Reference: Barry (2017)

Table 26 shows the single application rate (unit: pound per active ingredient per acre [lb AI/acre]) grouping of CPF products registered in California. This table is adapted from the US EPA spray drift exposure assessment document (Dawson et al., 2012). Application rates were used for translating the drift fraction outputs of AgDRIFT and AGDISP models into exposure estimates.

Table 26. Application Rates Grouping of Chlorpyrifos Usages in California

Single Application [a] (lb AI/acre)	**Example Use Site**	**Example Product**	**Comments**
6[b,c]	citrus fruits	Nufos 4E	Permitted use to control California red scale in Fresno, Tulare, Kern, Kings & Madera Counties only
4[b]	citrus fruits	Vulcan	Not specific to California
2.3	citrus fruits	Lorsban Advanced	Control of Citrus Psylla in California
2	tree fruits (e.g., apple), broccoli	Warhawk	Not specific to California
1	alfalfa, corn, cotton	Chlorpyrifos 4E AG	Not specific to California

[a] Modified from Dawson et al. (2012).
[b] Application rate of >2.3 lb AI/acre is not allowed for aerial equipment.
[c] An application rate higher than 6 lb AI/acre (i.e., 8 lb AI/acre) is identified in one product for use in pre-plant soil treatment. Because of the assumption employed for estimating inhalation exposure (i.e., ground based method results in the same air concentrations from aerial method at a the same ground-based application rate) and because of a much lower maximum aerial application allowed (i.e., 2.3 lb A.I./acre), exposure assessment based on 8 lb AI/acre application rate would greatly exaggerate the health risk estimated and, therefore, is not included in this exposure assessment. However, this application rate will be included in the future exposure assessment once the method of assessing inhalation exposure from the ground-based application methods is refined.

Evaluation of dermal and inhalation exposures of non-occupational/residential bystanders to spray drift was based on a modified US EPA residential SOP which incorporated off-site movement of pesticide from the results of AgDRIFT and AGDISP models (US EPA, 2013). Briefly, non-occupational/residential bystander exposure to spray drift is built on the assumption that CPF application may occur near residential sites or areas (e.g., schools) that the general public routinely access. Accordingly, the bystander exposures could occur indirectly via contact (e.g., dermal exposure) with the areas contaminated with the spray drift deposit and via inhalation of the airborne materials (e.g., aerosol) that may be transported off-site beyond the labeled buffer zone distance. It is important to note that direct exposures (via inhalation or

dermal contact) are prohibited by the product labels. Additionally, the California Code of Regulation §6614 also makes any direct exposure to humans a violation that may result in legal actions by the county or the State. DPR risk assessments only address legal application scenarios.

For assessing indirect exposure to spray drift for adults and small children, the US EPA residential lawns/turf post-application SOP is considered as the standard method (US EPA, 2013). That is, activities of adults and children on the contaminated lawn may result in transfer of spray drift deposition from different surfaces to their skin. In addition to the contact exposure via skin, exposure to spray drift deposition may occur via different mouthing activities, such as hand-to-mouth, object-to-mouth, and incidental soil ingestion for small children. In this exposure assessment, females 13-49 years old are a primary focus because of their potential increase in susceptibility to the toxicological effects of CPF during pregnancy. For children 1-2 years old, the US EPA Residential SOP (Addenda 1: Consideration of Spray Drift) indicates that children at this lifestage exhibit the highest exposure potential to pesticide contaminated lawn from spray drift due to dermal contact and different mouthing activities: hand-to-mouth, object-to-mouth, and incidental soil ingestion.

For estimating the dermal exposure from contaminated lawn, the following equation is employed.

$$\text{Dermal Dose} = \frac{\text{TTR} \times \text{TC} \times \text{ED} \times \text{AF} \times \text{CF}}{\text{BW}}$$

where:
TTR: turf transferable residue (μg/cm^2)
TC: transfer coefficient (cm^2/hr): 180000 for adults and 49000 for children
ED: exposure duration (hr/day): 1.5 for both adults and children
AF: absorption factor (dermal): 1 for computational purpose
CF: conversion factor of 0.001 mg/μg
BW: body weight (kg): 70 kg for females 13-49 years old; 13 kg for 1-2 years old (Andrews and Patterson, 2000)

According to the 2012 US EPA residential SOP, chemical-specific TTR on the day of application ($TTR_{Day\ 0}$) should be used for assessing individual exposure of pesticide on turf if available. A TTR study on CPF was conducted in three states including California, and the mean TTR values on the day of application were 0.124 μg/cm^2 in California and 0.12 μg/cm^2 as an average of the three states (Stafford and Robb, 1999).

Using the results of TTR study conducted in California (TTR_{expt}) (i.e., California-specific value), $TTR_{Day\ 0}$ for use in the drift exposure assessment can be estimated using the following equation:

$$\text{TTR}_{\text{Day 0}} = \left(\frac{\text{TTR}_{\text{expt}} \times \text{AppRate}_{\text{target}}}{\text{AppRate}_{\text{expt}}}\right) \times \text{F}$$

where:
TTR_{expt}: Experimentally measured mean turf transferable residue (μg/cm^2) of CPF in California (Dawson et al., 2012)

$AppRate_{expt}$: CPF application rate employed in the CA study (3.8 lb AI/A)
$AppRate_{target}$: CPF application rate(s) employed for assessing drift exposure
F: Fraction of nominal application rate (e.g., 6, 4, 2.3, 2, or 1 lb AI/acre) produced by AgDRIFT or AGDISP models as transferable residue following application

For estimating exposures to spray drift horizontal deposition through mouthing activities of small children (i.e., hand-to-mouth, object-to-mouth, and incidental soil ingestion), computational methods as defined in the US EPA residential SOP were strictly followed (US EPA, 2012). Hence, these computational methods are not reproduced in this exposure assessment.

For evaluating the inhalation exposure, breathing zone exposure concentrations of CPF in adults and small children are needed for the three application types: aerial, ground boom, and airblast. However, the empirical nature of the modules in the AgDRIFT for ground boom and airblast precludes the estimation of the needed breathing zone air concentrations. Accordingly, inhalation exposure calculations for all scenarios were performed using CPF air concentrations estimated using AGDISP.

IV.A.2.c. Spray Drift Exposure Estimates

V.A.2.c.i. Aerial Applications

Tables 27 and 28 show the drift deposition exposure (in μg/kg/day) and inhalation exposure estimates (as 1 hour time-weighted average air concentrations in mg/m^3) of CPF for children 1-2 years old and females 13-49 years old, respectively, due to aerial applications at two application volumes and three application rates with two types of aircraft: fixed-wing (AT802A airplane) and rotor-wing (Bell 205 helicopter). As can be seen in Tables 25 and 26, increases in CPF application rate resulted in a corresponding increase in the spray drift exposure estimates (regardless of the exposure route) at different distances downwind from the edge of the treated field.

For aerial applications, some CPF-containing products specify a minimum spray volume of not less than 2 gallons per acre (GPA). However, there appears to be no maximum spray volume specified. To evaluate the effect of spray volume on the horizontal deposition and inhalation exposure estimates, additional AGDISP simulations were performed. For a given application rate, the dermal exposure estimates are lower for the higher spray volume and the estimated 1 hour time-weighted average air concentrations increase with the spray volume. Further discussion of the effect of spray volume on the air concentrations of CPF can be found in (Barry, 2017) (Appendix 2).

Table 27. Dermal and Oral Doses and Inhalation Concentration for Children (1-2 years old) at Various Distances Downwind from the Fields Treated with CPF by Aircraft or Helicopter

Application Scenario	Appl. Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	Dose at Various Distance Downwind from the Treated Fields (µg/kg/d)						
				10 feet	25 feet[c]	50 feet	100 feet	250 feet	500 feet	1000 feet
Dermal and Oral Exposure: Aircraft or Helicopter (Children 1-2 years old)										
AT802A Fixed Wing Aircraft	2[a]	Dermal	1	35.46	30.24	23.80	16.03	8.36	4.98	2.66
			2	71.18	60.51	47.45	31.70	15.87	8.63	3.39
			2.3	81.85	69.55	54.48	36.32	18.16	9.63	3.73
		Object-to-Mouth	1	0.023	0.019	0.015	0.010	0.005	0.003	0.002
			2	0.046	0.039	0.030	0.020	0.010	0.006	0.002
			2.3	0.052	0.044	0.035	0.023	0.012	0.006	0.002
		Hand-to-Mouth	1	0.738	0.629	0.495	0.334	0.174	0.104	0.055
			2	1.481	1.259	0.987	0.659	0.330	0.180	0.071
			2.3	1.703	1.447	1.134	0.756	0.378	0.200	0.078
		Soil Ingestion	1	0.0055	0.0047	0.0037	0.0025	0.0013	0.0008	0.0004
			2	0.0111	0.0094	0.0074	0.0049	0.0025	0.0013	0.0005
			2.3	0.0127	0.0108	0.0085	0.0056	0.0028	0.0015	0.0006
Bell 205 Helicopter	2	Dermal	1	45.28	28.65	17.55	10.66	6.81	4.04	1.97
			2	91.18	58.08	35.76	22.25	12.32	6.31	2.77
			2.3	104.90	66.83	41.16	25.67	13.92	7.00	3.01
		Object-to-Mouth	1	0.0289	0.0183	0.0112	0.0068	0.0043	0.0026	0.0013
			2	0.0582	0.0371	0.0228	0.0142	0.0079	0.0040	0.0018
			2.3	0.0670	0.0427	0.0263	0.0164	0.0089	0.0045	0.0019
		Hand-to-Mouth	1	0.9419	0.5961	0.3650	0.2219	0.1416	0.0841	0.0411
			2	1.897	1.208	0.744	0.463	0.256	0.131	0.058
			2.3	2.182	1.390	0.856	0.534	0.290	0.146	0.063
		Soil Ingestion	1	0.0070	0.0044	0.0027	0.0017	0.0011	0.0006	0.0003
			2	0.0142	0.0090	0.0056	0.0035	0.0019	0.0010	0.0004
			2.3	0.0163	0.0104	0.0064	0.0040	0.0022	0.0011	0.0005
AT802A Fixed Wing Aircraft	15[b]	Dermal	1	30.83	26.00	20.79	13.91	7.14	4.43	3.30
			2	64.13	54.32	43.76	29.81	15.68	10.00	7.27
			2.3	74.05	62.80	50.67	34.50	18.20	11.58	8.40
		Object-to-Mouth	1	0.020	0.017	0.013	0.009	0.005	0.003	0.002
			2	0.041	0.035	0.028	0.019	0.010	0.006	0.005
			2.3	0.047	0.040	0.032	0.022	0.012	0.007	0.005
		Hand-to-Mouth	1	0.64	0.54	0.43	0.29	0.15	0.09	0.07
			2	1.33	1.13	0.91	0.62	0.33	0.21	0.15

			2.3	1.54	1.31	1.05	0.72	0.38	0.24	0.17
		Soil Ingestion	1	0.005	0.004	0.003	0.002	0.001	0.001	0.001
			2	0.010	0.008	0.007	0.005	0.002	0.002	0.001
			2.3	0.011	0.010	0.008	0.005	0.003	0.002	0.001
Bell 205 Helicopter	15	Dermal	1	42.08	25.88	15.02	8.71	6.05	4.54	2.97
			2	86.45	53.91	32.10	19.00	13.28	9.45	5.72
			2.3	99.93	62.46	37.30	22.15	15.36	10.78	6.53
		Object-to-Mouth	1	0.027	0.017	0.010	0.006	0.004	0.003	0.002
			2	0.055	0.034	0.021	0.012	0.008	0.006	0.004
			2.3	0.064	0.040	0.024	0.014	0.010	0.007	0.004
		Hand-to-Mouth	1	0.88	0.54	0.31	0.18	0.13	0.09	0.06
			2	1.80	1.12	0.67	0.40	0.28	0.20	0.12
			2.3	2.08	1.30	0.78	0.46	0.32	0.22	0.14
		Soil Ingestion	1	0.007	0.004	0.002	0.001	0.001	0.001	0.000
			2	0.013	0.008	0.005	0.003	0.002	0.001	0.001
			2.3	0.016	0.010	0.006	0.003	0.002	0.002	0.001
1-Hour Air Concentration at Various Distance Downwind from the Treated Fields (mg/m^3)										
				10 feet	**25 feet**	**50 feet**	**100 feet**	**250 feet**	**500 feet**	**1000 feet**
AT802A	2	Inhalation	1	0.0318	0.0292	0.0264	0.022	0.0161	0.0117	0.0065
			2	0.0546	0.0493	0.0437	0.0350	0.0237	0.0153	0.0072
			2.3	0.0583	0.0526	0.0464	0.0371	0.0250	0.0159	0.0075
	15	Inhalation	1	0.0443	0.0413	0.0391	0.0348	0.0289	0.0243	0.0190
			2	0.0758	0.0703	0.0660	0.0579	0.0468	0.0381	0.0279
			2.3	0.0841	0.0779	0.0730	0.0637	0.0513	0.0415	0.0299
Bell 205 Helicopter	2	Inhalation	1	0.0409	0.0336	0.0274	0.0219	0.0153	0.0102	0.0058
			2	0.0728	0.0580	0.0458	0.0345	0.0215	0.0130	0.0068
			2.3	0.0771	0.0611	0.0482	0.0362	0.0222	0.0133	0.0069
	15	Inhalation	1	0.0685	0.0592	0.0517	0.0448	0.0367	0.0288	0.0202
			2	0.0967	0.0828	0.0715	0.0612	0.0488	0.0373	0.0252
			2.3	0.1074	0.0917	0.0789	0.0671	0.0532	0.0402	0.0269

[a] Minimum spray volume as specified on some CPF product labels for the aerial application.
[b] Spray volume of 15 GPA is chosen in exercise for illustrative purpose.
[c] Buffer zone of 25 feet is required for aerial application of CPF.

Table 28. Estimated Dermal Doses and Inhalation Concentrations for Females (13-49 years old) at Various Distances from a Field Treated with Chlorpyrifos Using Aerial Equipment

Aircraft	Spray Volume (gallon/acre)	Application Rate (lb/acre)	Dose at Various Distance Downwind from the Treated Fields (µg/kg/day)						
			10 (feet)	25 (feet)[c]	50 (feet)	100 (feet)	250 (feet)	500 (feet)	1000 (feet)
AT802A	2[a]	1	24.19	20.63	16.24	10.94	5.70	3.40	1.81
		2	48.56	41.28	32.37	21.62	10.82	5.89	2.32
		2.3	55.84	47.45	37.17	24.78	12.39	6.57	2.55
	15[b]	1	21.03	17.73	14.18	9.49	4.87	3.02	2.25
		2	43.75	37.05	29.86	20.34	10.70	6.82	4.96
		2.3	50.52	42.84	34.56	23.54	12.42	7.90	5.73
Bell 205 Helicopter	2[a]	1	30.89	19.55	11.97	7.27	4.64	2.76	1.35
		2	62.20	39.62	24.39	15.18	8.41	4.30	1.89
		2.3	71.56	45.59	28.08	17.51	9.50	4.78	2.06
	15[b]	1	28.71	17.66	10.25	5.94	4.13	3.10	2.03
		2	58.98	36.78	21.90	12.96	9.06	6.44	3.90
		2.3	68.17	42.61	25.45	15.11	10.48	7.35	4.46
1-Hour Air Concentration at Various Distance Downwind from the Treated Fields (mg/m^3)									
			10 (feet)	**25 (feet)**	**50 (feet)**	**100 (feet)**	**250 (feet)**	**500 (feet)**	**1000 (feet)**
AT802A	2	1	0.0234	0.0218	0.0194	0.0163	0.0118	0.0085	0.0047
		2	0.0399	0.0367	0.0320	0.0259	0.0174	0.0111	0.0052
		2.3	0.0428	0.0394	0.0341	0.0275	0.0183	0.0115	0.0054
	15	1	0.0323	0.0306	0.0287	0.0256	0.0212	0.0177	0.0138
		2	0.0553	0.0522	0.0484	0.0426	0.0342	0.0278	0.0202
		2.3	0.0614	0.0579	0.0536	0.0469	0.0375	0.0303	0.0217
Bell 205 Helicopter	2[a]	1	0.0288	0.0240	0.0197	0.0158	0.0111	0.0074	0.0042
		2	0.0500	0.0404	0.0322	0.0246	0.0154	0.0093	0.0049
		2.3	0.0538	0.0435	0.0345	0.0260	0.0160	0.0096	0.0050
	15[b]	1	0.0487	0.0426	0.0373	0.0325	0.0266	0.0209	0.0147
		2	0.0686	0.0596	0.0516	0.0443	0.0353	0.0270	0.0183
		2.3	0.0762	0.0659	0.0569	0.0485	0.0385	0.0291	0.0195

[a] Minimum spray volume as specified on some CPF product labels for the aerial application.
[b] Spray volume of 15 GPA is chosen in exercise for illustrative purpose.
[c] Buffer zone of 25 feet is required for aerial application of CPF.

IV.A.2.c.ii. Ground-Based Applications

Table 29 shows the drift deposition exposure estimates (in μg/kg/day) of CPF for females 13-49 years old at four allowable application rates with two ground-based application methods, ground boom and airblast. For ground boom, spray drift deposition estimates were derived using two swath percentiles: 50th and 90th percentiles (see Appendix 2). Tables 30 and 31 show the spray drift exposure estimates of chlorpyrifos for children 1-2 years old: ground boom 90th percentile and ground boom 50th percentile deposition estimates. Table 32 shows the spray drift exposure estimates of chlorpyrifos for children 1-2 years old for orchard airblast. As expected for both ground boom and orchard airblast application methods and population subgroups, the spray drift exposure estimates increase with the application rates of chlorpyrifos. The higher horizontal deposition exposure estimates of the high-boom compared with the low-boom are 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 of orchard airblast compared to ground boom are consistent with the much finer droplet spectrum of airblast sprayer application and the upward direction by the airblast sprayer of fine spray into the orchard canopy. Table 33 shows the drift inhalation concentration estimate (in mg/m3) for both children 1-2 years old (1.7 ft height) and females 13-49 years old (5 ft height).

Table 29. Estimated Dermal Doses for Females (13-49 years old) at Various Distances from a Field Treated with Chlorpyrifos Using Ground-Based Equipment: Ground Boom and Airblast

Application Scenarios	Swaths (Percentile)	Appl. Rate (lb/acre)	Dose at Various Distance Downwind from the Treated Fields (μg/kg/day)						
			25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Ground boom									
High boom	40 (50th)[a]	1	1.1957	0.7929	0.5916	0.4657	0.3398	0.2643	0.2140
		2	2.3914	1.5859	1.1831	0.9314	0.6797	0.5286	0.4279
		4	4.7829	3.1718	2.3663	1.8628	1.3593	1.0573	0.8559
		6	7.1743	4.7577	3.5494	2.7942	2.0390	1.5859	1.2838
High boom	40 (90th)[a]	1	1.6992	1.2209	0.9440	0.7552	0.5664	0.4531	0.3776
		2	3.3983	2.4418	1.8880	1.5104	1.1328	0.9062	0.7552
		4	6.7967	4.8835	3.7759	3.0208	2.2656	1.8125	1.5104
		6	10.1950	7.3253	5.6639	4.5311	3.3983	2.7187	2.2656
Low boom	40 (50th)[a]	1	0.6293	0.4279	0.3272	0.2517	0.1888	0.1510	0.1259
		2	1.2586	0.8559	0.6545	0.5035	0.3776	0.3021	0.2517
		4	2.5173	1.7118	1.3090	1.0069	0.7552	0.6042	0.5035
		6	3.7759	2.5676	1.9635	1.5104	1.1328	0.9062	0.7552
Low boom	40 (90th)[a]	1	1.0699	0.7804	0.6042	0.4909	0.3650	0.3021	0.2517
		2	2.1397	1.5607	1.2083	0.9817	0.7300	0.6042	0.5035
		4	4.2794	3.1214	2.4166	1.9635	1.4600	1.2083	1.0069
		6	6.4191	4.6822	3.6249	2.9452	2.1900	1.8125	1.5104
Orchard Airblast									
Dormant Apples	60	1	6.9666	2.6507	1.3002	0.7388	0.3121	0.1649	0.0994
		2	13.9332	5.3014	2.6004	1.4777	0.6243	0.3298	0.1989
		4	27.8664	10.6028	5.2007	2.9553	1.2486	0.6595	0.3977
		6	41.7997	15.9043	7.8011	4.4330	1.8729	0.9893	0.5966
Sparse Orchard	60	1	5.6488	2.5727	1.4449	0.9226	0.4695	0.2832	0.1901
		2	11.2976	5.1454	2.8899	1.8452	0.9390	0.5664	0.3801
		4	22.5952	10.2907	5.7797	3.6904	1.8779	1.1328	0.7602
		6	33.8928	15.4360	8.6696	5.5355	2.8169	1.6992	1.1403

a-Horizontal deposition estimates were derived using a 50th percentile or 90th percentile horizontal deposition.

Table 30. Estimated Dermal and Mouthing Doses for Children (1-2 years old) at Various Distances from a Field Treated with Chlorpyrifos Using Ground Boom Equipment (High Boom)

Scenarios	Swaths (percentile)	Exposure Route	Appl. Rate (lb/acre)	Dose at Various Distance Downwind from the Treated Fields (µg/kg/day)						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
High boom	40 (50^{th})[a]	Dermal	1	1.7527	1.1623	0.8671	0.6826	0.4981	0.3874	0.3136
			2	3.5054	2.3246	1.7342	1.3653	0.9963	0.7749	0.6273
			4	7.0108	4.6492	3.4685	2.7305	1.9925	1.5497	1.2546
			6	10.5162	6.9739	5.2027	4.0958	2.9888	2.3246	1.8818
		Object-to-Mouth	1	0.0011	0.0007	0.0006	0.0004	0.0003	0.0002	0.0002
			2	0.0022	0.0015	0.0011	0.0009	0.0006	0.0005	0.0004
			4	0.0045	0.0030	0.0022	0.0017	0.0013	0.0010	0.0008
			6	0.0067	0.0045	0.0033	0.0026	0.0019	0.0015	0.0012
		Hand-to-Mouth	1	0.0365	0.0242	0.0180	0.0142	0.0104	0.0081	0.0065
			2	0.0729	0.0484	0.0361	0.0284	0.0207	0.0161	0.0130
			4	0.1459	0.0967	0.0722	0.0568	0.0415	0.0322	0.0261
			6	0.2188	0.1451	0.1082	0.0852	0.0622	0.0484	0.0391
		Soil Ingestion	1	0.0003	0.0002	0.0001	0.0001	0.0001	0.0001	0.00005
			2	0.0005	0.0004	0.0003	0.0002	0.0002	0.0001	0.0001
			4	0.0011	0.0007	0.0005	0.0004	0.0003	0.0002	0.0002
			6	0.0016	0.0011	0.0008	0.0006	0.0005	0.0004	0.0003
High boom	40 (90^{th})[a]	Dermal	1	2.4907	1.7896	1.3837	1.1070	0.8302	0.6642	0.5535
			2	4.9813	3.5792	2.7674	2.2139	1.6604	1.3284	1.1070
			4	9.9627	7.1584	5.5348	4.4279	3.3209	2.6567	2.2139
			6	14.9440	10.7375	8.3022	6.6418	4.9813	3.9851	3.3209
		Object-to-Mouth	1	0.0016	0.0011	0.0009	0.0007	0.0005	0.0004	0.0004
			2	0.0032	0.0023	0.0018	0.0014	0.0011	0.0008	0.0007
			4	0.0064	0.0046	0.0035	0.0028	0.0021	0.0017	0.0014
			6	0.0095	0.0069	0.0053	0.0042	0.0032	0.0025	0.0021
		Hand-to-Mouth	1	0.0518	0.0372	0.0288	0.0230	0.0173	0.0138	0.0115
			2	0.1036	0.0745	0.0576	0.0461	0.0345	0.0276	0.0230
			4	0.2073	0.1489	0.1151	0.0921	0.0691	0.0553	0.0461
			6	0.3109	0.2234	0.1727	0.1382	0.1036	0.0829	0.0691
		Soil Ingestion	1	0.0004	0.0003	0.0002	0.0002	0.0001	0.0001	0.0001
			2	0.0008	0.0006	0.0004	0.0003	0.0003	0.0002	0.0002
			4	0.0015	0.0011	0.0009	0.0007	0.0005	0.0004	0.0003
			6	0.0023	0.0017	0.0013	0.0010	0.0008	0.0006	0.0005

a-Horizontal deposition estimates were derived using a 50^{th} percentile or 90^{th} percentile horizontal deposition.

Table 31. Estimated Dermal and Mouthing Doses for Children (1-2 years old) at Various Distances from a Field-Treated with Chlorpyrifos Using Ground Boom Equipment (Low Boom)

Scenarios	Swaths (percentile)	Exposure Route	Appl. Rate (lb/acre)	Dose at Various Distance Downwind from the Treated Fields (µg/kg/day)						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Low boom	40 (50th)[a]	Dermal	1	0.9225	0.6273	0.4797	0.3690	0.2767	0.2214	0.1845
			2	1.8449	1.2546	0.9594	0.7380	0.5535	0.4428	0.3690
			4	3.6899	2.5091	1.9187	1.4760	1.1070	0.8856	0.7380
			6	5.5348	3.7637	2.8781	2.2139	1.6604	1.3284	1.1070
		Object-to-Mouth	1	0.0006	0.0004	0.0003	0.0002	0.0002	0.0001	0.0001
			2	0.0012	0.0008	0.0006	0.0005	0.0004	0.0003	0.0002
			4	0.0024	0.0016	0.0012	0.0009	0.0007	0.0006	0.0005
			6	0.0035	0.0024	0.0018	0.0014	0.0011	0.0008	0.0007
		Hand-to-Mouth	1	0.0192	0.0130	0.0100	0.0077	0.0058	0.0046	0.0038
			2	0.0384	0.0261	0.0200	0.0154	0.0115	0.0092	0.0077
			4	0.0768	0.0522	0.0399	0.0307	0.0230	0.0184	0.0154
			6	0.1151	0.0783	0.0599	0.0461	0.0345	0.0276	0.0230
		Soil Ingestion	1	0.0001	0.0001	0.0001	0.0001	0.0000	0.0000	0.0000
			2	0.0003	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001
			4	0.0006	0.0004	0.0003	0.0002	0.0002	0.0001	0.0001
			6	0.0009	0.0006	0.0004	0.0003	0.0003	0.0002	0.0002
Low boom	40 (90th)[a]	Dermal	1	1.5682	1.1439	0.8856	0.7195	0.5350	0.4428	0.3690
			2	3.1364	2.2877	1.7711	1.4391	1.0701	0.8856	0.7380
			4	6.2728	4.5754	3.5423	2.8781	2.1401	1.7711	1.4760
			6	9.4092	6.8632	5.3134	4.3172	3.2102	2.6567	2.2139
		Object-to-Mouth	1	0.0010	0.0007	0.0006	0.0005	0.0003	0.0003	0.0002
			2	0.0020	0.0015	0.0011	0.0009	0.0007	0.0006	0.0005
			4	0.0040	0.0029	0.0023	0.0018	0.0014	0.0011	0.0009
			6	0.0060	0.0044	0.0034	0.0028	0.0021	0.0017	0.0014
		Hand-to-Mouth	1	0.0326	0.0238	0.0184	0.0150	0.0111	0.0092	0.0077
			2	0.0652	0.0476	0.0368	0.0299	0.0223	0.0184	0.0154
			4	0.1305	0.0952	0.0737	0.0599	0.0445	0.0368	0.0307
			6	0.1957	0.1428	0.1105	0.0898	0.0668	0.0553	0.0461
		Soil Ingestion	1	0.0002	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001
			2	0.0005	0.0004	0.0003	0.0002	0.0002	0.0001	0.0001
			4	0.0010	0.0007	0.0005	0.0004	0.0003	0.0003	0.0002
			6	0.0015	0.0011	0.0008	0.0007	0.0005	0.0004	0.0003

a-Horizontal deposition estimates were derived using a 50th percentile or 90th percentile horizontal deposition

Table 32. Estimated Dermal and Mouthing Doses for Children (1-2 years old) at Various Distances from Chlorpyrifos Treated Apple Orchards

Scenarios	Swaths	Exposure Route	Appl. Rate (lb/acre)	Dose at Various Distance Downwind from the Treated Fields (µg/kg/day)						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Dormant Apple	60	Dermal	1	10.2117	3.8854	1.9058	1.0830	0.4575	0.2417	0.1458
			2	20.4235	7.7709	3.8116	2.1660	0.9151	0.4834	0.2915
			4	40.8470	15.5418	7.6233	4.3319	1.8302	0.9667	0.5830
			6	61.2704	23.3127	11.4349	6.4979	2.7453	1.4501	0.8745
		Object-to-Mouth	1	0.0065	0.0025	0.0012	0.0007	0.0003	0.0002	0.0001
			2	0.0130	0.0050	0.0024	0.0014	0.0006	0.0003	0.0002
			4	0.0261	0.0099	0.0049	0.0028	0.0012	0.0006	0.0004
			6	0.0391	0.0149	0.0073	0.0041	0.0018	0.0009	0.0006
		Hand-to-Mouth	1	0.2124	0.0808	0.0396	0.0225	0.0095	0.0050	0.0030
			2	0.4249	0.1617	0.0793	0.0451	0.0190	0.0101	0.0061
			4	0.8498	0.3233	0.1586	0.0901	0.0381	0.0201	0.0121
			6	1.2747	0.4850	0.2379	0.1352	0.0571	0.0302	0.0182
		Soil Ingestion	1	0.0016	0.0006	0.0003	0.0002	0.0001	0.0000	0.0000
			2	0.0032	0.0012	0.0006	0.0003	0.0001	0.0001	0.0000
			4	0.0063	0.0024	0.0012	0.0007	0.0003	0.0002	0.0001
			6	0.0095	0.0036	0.0018	0.0010	0.0004	0.0002	0.00014
Sparse Orchard	60	Dermal	1	8.2801	3.7711	2.1180	1.3523	0.6882	0.4151	0.2786
			2	16.5602	7.5421	4.2360	2.7047	1.3763	0.8302	0.5572
			4	33.1203	15.0842	8.4720	5.4094	2.7526	1.6604	1.1143
			6	49.6805	22.6263	12.7079	8.1140	4.1290	2.4907	1.6715
		Object-to-Mouth	1	0.0053	0.0024	0.0014	0.0009	0.0004	0.0003	0.0002
			2	0.0106	0.0048	0.0027	0.0017	0.0009	0.0005	0.0004
			4	0.0212	0.0096	0.0054	0.0035	0.0018	0.0011	0.0007
			6	0.0317	0.0145	0.0081	0.0052	0.0026	0.0016	0.0011
		Hand-to-Mouth	1	0.1723	0.0785	0.0441	0.0281	0.0143	0.0086	0.0058
			2	0.3445	0.1569	0.0881	0.0563	0.0286	0.0173	0.0116
			4	0.6890	0.3138	0.1763	0.1125	0.0573	0.0345	0.0232
			6	1.0336	0.4707	0.2644	0.1688	0.0859	0.0518	0.0348
		Soil Ingestion	1	0.0013	0.0006	0.0003	0.0002	0.0001	0.0001	0.0000
			2	0.0026	0.0012	0.0007	0.0004	0.0002	0.0001	0.0001
			4	0.0051	0.0023	0.0013	0.0008	0.0004	0.0003	0.0002
			6	0.0077	0.0035	0.0020	0.0013	0.0006	0.0004	0.00026

Table 33. Estimated Air Concentrations at Various Distances from a Field Treated with Chlorpyrifos Using Aerial Equipment

Aircraft	Spray Volume (gallon/acre)	Height of Air Concentration (ft)	Application Rate (lb/acre)	1-Hour Air Concentration at Various Distance Downwind from the Treated Fields[a] (mg/m^3)						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
AT802A	2	1.7 ft	1	0.0292	0.0264	0.0239	0.0220	0.0194	0.0175	0.0161
			2	0.0493	0.0437	0.0386	0.0350	0.0300	0.0264	0.0237
			4	0.0795	0.0688	0.0594	0.0526	0.0431	0.0367	0.0315
			6	0.1042	0.0884	0.0752	0.0650	0.0508	0.0414	0.0348
		5 ft	1	0.0218	0.0194	0.0176	0.0163	0.0143	0.0129	0.0118
			2	0.0367	0.0320	0.0285	0.0259	0.0221	0.0195	0.0174
			4	0.0596	0.0503	0.0439	0.0389	0.0319	0.0269	0.0230
			6	0.0781	0.0643	0.0550	0.0479	0.0377	0.0305	0.0253

a-These estimated doses are used as surrogate inhalation doses for orchard airblast and ground boom applications.

IV.A.2.d. Exposure from House Dust

Inhalation of airborne material, dermal contact with contaminated surfaces, and non-dietary oral ingestion (e.g., pica) are potential exposure of chlorpyrifos associated with spray drift. In addition to these outdoor post-application exposure pathways, exposure to chlorpyrifos may occur via incidental ingestion of contaminated indoor dust especially in young children in agricultural families (Buck et al., 1999; Quiros-Alcala et al., 2011). Prior to the restrictions of indoor chlorpyrifos use, house dust contained chlorpyrifos residues derived from the indoor chlorpyrifos applications (e.g., in home insect control) (Lewis et al., 2001) or from "take-home" exposure from occupational settings (Fenske et al., 2013; Smith et al., 2017). In 2000, US EPA heavily restricted indoor use of chlorpyrifos, leaving only roach baits in child resistant packaging registered for indoor use. Therefore, sources outside of the home can now be assumed to be the sole contributors to chlorpyrifos residues in house dust. Figure 11 shows the pounds of chlorpyrifos applied in California two years before and one year after the US EPA action. Also shown in Figure 11 is the maximum concentrations of chlorpyrifos measured on house dust samples collected from the same farmworker community at Salinas Valley, CA in 1999 and 2002 (Bradman et al., 2007; Harnly et al., 2009). Similar to the reduction in amounts of chlorpyrifos applied over the time period of 1999-2002, the maximum chlorpyrifos concentrations in house dust decreased from 9810 ng/g in 1999 to 1200 ng/g in 2002. Because these household dust samples were collected from homes of farmworkers within the same agricultural area, the substantial decrease (i.e., a factor of ~8) in the maximum house dust concentrations over this time period suggests that the indoor uses may have been the major source of chlorpyrifos in contaminated house dust. In other words, after the restrictions of home use, outdoor sources such as "take-home" by farmworkers from their occupations become the dominant source of chlorpyrifos in house dust in these agricultural families.

Studies showed that chlorpyrifos concentrations in house dust are higher in farmworker homes than non-farmworker homes (Smith et al., 2017). Accordingly, assessing 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. For evaluating children's exposure to chlorpyrifos via house dust, this assessment employs house dust concentration of chlorpyrifos after the indoor use cancellation. Specifically, in the study by (Bradman *et al.*, 2007), organophosphate pesticides including chlorpyrifos were measured in house dust samples collected from 20 farmworker families in 2002 at Salinas Valley, CA. Combining the highest measured chlorpyrifos house dust concentration (i.e., 1200 ng/g) with a daily dust ingestion rate for children 0 - 2 years old (i.e., 304 mg/day [at the 95th percentile]) (OEHHA, 2012), and assuming an infant body (i.e., <1 yr old) weight of 7.6 kg (Andrews and Patterson, 2000), and 100% oral absorption, a short term absorbed daily dose can be estimated as 0.048 μg/kg/day.

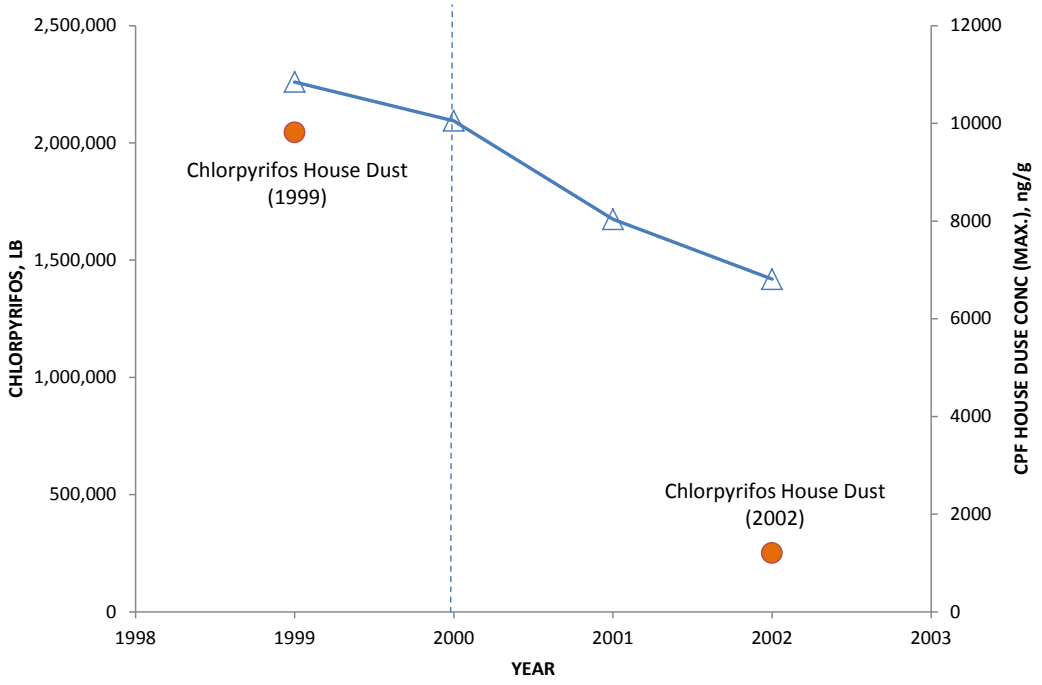


Figure 11. Pounds of chlorpyrifos applied in California from 1999 to 2002 and maximum concentrations of chlorpyrifos measured in house dust samples collected from Salinas Valley, CA in 1999 and 2002
(Bradman et al., 2007; Harnly et al., 2009)

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

Below is a brief description of the CPF dietary (food only) and drinking water (DW: refined, ground water and surface water) risk assessment for California. The subpopulations of concern for both dietary (food only) and DW acute and steady-state exposures were infants (< 1 year old), children (1-2 years old), children (6-12 years old), and females (13-49 years old). The PoDs for these subgroups were presented in the 2014 US EPA Revised Human Health Risk Assessment for CPF (2014a) and in the Hazard Identification, above.

IV.B.1. Food-Only Exposure Assessment

IV.B.1.a. Summary of the 2014 US EPA Food-Only Exposure Assessment

Acute food-only exposures were calculated for every standard subpopulation and steady-state exposures were calculated for four sentinel subpopulations identified in the US EPA risk assessment: infants (< 1 year old), children 1-2 years, children 6-12 years, and females 13-49 years (US EPA, 2014b).

IV.B.2. Description of Dietary Exposure Assessment Models

1) DEEM-FCID

DEEM-FCID is a computer program for estimating exposure and/or risk to human health from pesticides in food (US EPA, 2015). The software incorporates food consumption data from the National Health and Nutrition Examination Survey/"What We Eat in

America" (NHANES/WWEIA) dietary survey. Individual dietary consumption records reported in the survey are translated into more than 500 US EPA-defined food commodities using the Food Commodity Intake Database. Dietary consumption data, expressed in units of food commodities (kg food/kg body weight), are combined with pesticide residue data in a probabilistic analysis to estimate pesticide exposure levels. Exposure can be calculated for specific segments of the population based on age, gender, or ethnicity, and for periods of time corresponding to acute (≤ 1 day), chronic, or lifetime effects.

2) Calendex-FCID

Calendex-FCID is a component DEEM-FCID that allows the analysis of variations in exposure during the calendar year as well the ability to aggregate exposures from multiple routes and pathways, such as oral, dermal, and inhalation exposures resulting from residues in food as well as residential and/or occupational exposure. In US EPA's 2014 dietary exposure assessment, Calendex-FCID was used because it allowed the estimation of 21-day average dietary exposure, which corresponded to the period of time required for steady-state cholinesterase inhibition by CPF (US EPA, 2015).

IV.B.3. Residue Data and Refinements

CPF is used on a wide variety of food crops, including some of the most important commodities in California. Based on the most recent five years of use data (2011-2015), the top agricultural uses in the state were almond, alfalfa, walnut, orange, and cotton. Average annual use for all sites, including all agricultural and non-agricultural uses, was 1.3 million lbs/year.

US EPA tolerances for residues of CPF are presently established on a large number of crops. There are 79 individual tolerances and three crop group tolerances ranging from 0.1 to 20 ppm (CFR 40 §180.342, updated August 12, 2015). Two of the tolerances, for grape and asparagus, are regional. CPF-oxon residues are not included in the tolerances established for CPF residues because it is generally not found in food. US EPA's 2014 dietary exposure assessment incorporated the latest residue data from USDA's Pesticide Data Program (PDP) (through 2012) and updated usage information (2004-2012). Steady-state exposure was analyzed as a 21-day rolling average throughout the year. The assessment used an extensive set of processing factors including those for cooking and peeling, as well as default factors for dried or juice food types. The factors from the cooking study were summarized in the 2011 preliminary dietary exposure assessment.

The metabolite CPF-oxon was not included in the food-only exposure assessment because field trial and metabolism studies showed that it was not present in crops. Also, it was not detected by the PDP program from 2007 through 2012, except in one potato sample. CPF in not registered for use on potatoes in the US (US EPA, 2014b).

Seventy residue data files were used in the probabilistic analysis. The same data files were used in the acute and steady state exposure assessments. For crops not sampled by PDP, data were translated from similar crops where appropriate. The following commodities had no detects of

CPF residues: sugar beet; dried peas and beans; dried peach, banana, and plantain; field corn; popcorn; sorghum (syrup); triticale and wheat flour; sunflower; cottonseed; most meat, milk and egg food types; fig; peanut; peppermint; and spearmint. For those commodities, US EPA's analysis used anticipated residues, tolerance values, or point estimates of residues, depending on consumption rate of the commodity, and the availability of either field trial data or residue data from similar commodities.

Acute exposures were calculated for the general US population and eight subpopulations: infants, children 1-2 years, children 3-5 years, children 6-12 years, youth 13-19 years, adults 20-49 years, adults 50-99 years, and females 13-49 years. Steady state exposures were calculated for four sentinel populations characterized in the PBPK-PB model: infants, children 1-2 years, children 6-12 years, and females 13-49 years.

The 2014 US EPA exposure values were estimated on a per capita basis (all individuals surveyed). HHA selects per user-day basis (consumers only or the population that is exposed) for the acute exposure rather than the entire population (per capita) (CDPR, 2009). In many exposure scenarios, per capita risks would be lower than per user risks. Therefore, HHA conducted a sensitivity analysis of food consumption by infant population subgroups in DEEM-FCID v3.16 to determine if consumption was significantly different among them. Residue levels for all commodities excluding water, was set at 1 ppm (point estimate). Table 34 shows the number of users compared to number of persons surveyed in each population subgroup. Because so many commodities were included, most persons surveyed were users. The exposure estimates at the 95th percentile were slightly higher for non-nursing infants compared to all infants, but at the 99.9th percentile, the exposure estimates for non-nursing infants and all infants were essentially the same. Nevertheless, we recognize that non-nursing infants on formula can have higher exposures to CPF on average, but at the higher exposure levels the difference in exposure estimates between non-nursing infants and all infants is small.

Table 34. Comparison of Consumption of Food Commodities for Infant Population

Population Subgroup	Persons Surveyed	Users Surveyed	Exposure (mg/kg/day) per capita		
			Mean	95th percentile	99.9th percentile
Nursing infants	792	604	0.019639	0.069205	0.181581
Non-nursing infants	1708	1707	0.046784	0.125402	0.222562
All infants	2500	2311	0.038403	0.111445	0.221506

HHA also examined the potential for CPF exposure through formula or breast milk in infants. Infant formulas are prepared using heat and other purification procedures to reduce potential pesticide residues from application on crops used in formula ingredients. Infant formulas are mainly based on cow's milk or soy protein and soy oil. Monitoring studies over the years have confirmed that pesticides are rarely detected in infant formulas (National Research Council Committee on Pesticides in the Diets of Infants and Children, NRC, 1993). For CPF and CPF-oxon, PDP (2013 and 2014) analyzed 705 samples of cow milk and 706 samples of soy-based infant formula and found no detectable residues (LOD ranged from 0.001 and 0.01 ppm). PDP monitoring of cow's milk in 2012 resulted in 3 chlorpyrifos detects out of 792 samples (0.4%), with a LOD of 0.5 ppb.

Presently, there are very few studies that measured chlorpyrifos concentrations in breast milk of mothers in the US. A 2011 pilot study from the CHAMACOS Cohort measured chlorpyrifos concentrations in the breast milk of women residing in urban and agricultural regions in CA (Weldon et al., 2011). The study detected chlorpyrifos residues in breast milk in a relatively small number of subjects (21 urban women and 13 agricultural women) . The residues ranged from 13 to 1,000 pg/g milk. The median values between urban and agricultural women were similar (24.5 and 28.0 pg/g, respectively). The LOD's in this study were very low, ranging from 0.1-0.5 pg/g. In a study in India, Bedi *et al.* (2013) found much higher residues than in the Weldon study, although the LOD was not reported. The number of subjects was also relatively small (primiparate and 19 multiparate women). While not referring to this particular study by Bedi et al., Weldon and colleagues suggested a hypothesis that higher residues in breast milk from Indian women was associated with non-compliance of re-entry intervals after applications (Weldon et al., 2011). In a dissertation from the University of Tennessee (Casey, 2005), the author used ELISA to detect residues of chlorpyrifos in breast milk from mothers in Tennessee. This method has not been validated, although initial results were approximately 40 times higher in 26 lactating and 26 non-lactating females than levels reported in Weldon et al. (2011). The former has not yet been published as a peer-reviewed manuscript. Lastly, as mentioned earlier, PDP monitoring of cow's milk reported only 3 chlorpyrifos detects out of 792 samples with a LOD of 0.0005 ppm or 0.5 ppb (PDP 2015).

Taken as a whole these studies reported chlorpyrifos residues in breast milk, but the magnitude of them is uncertain. The Weldon *et al.* (2011) appears to be the most reliable estimate of breast milk residues in US women with the legal uses of chlorpyrifos and the residues were low. HHA will continue to follow the literature on pesticides residues in human milk and will evaluate children's exposure to chlorpyrifos via the lactational pathway as data become available.

Exposure estimates were compared to population-adjusted doses (PADs) from US EPA's evaluation. PADs were based on PoDs that were estimated from PBPK-PD modeling of RBC cholinesterase inhibition in humans.

IV.B.4. Results of Dietary (food-only) Exposure Assessment

Exposure estimates from the 2014 US EPA assessment are shown in Table 35 and Table 36. Children 1-2 years old were identified as the highest exposed population subgroup. At the 99.9th percentile, their exposure was estimated at 0.000423 mg/kg. Although a commodity contribution analysis was not included in either the 2011 or 2014 US EPA exposure assessments, residues in peaches, peppers, apples, plums, grapefruit juice, grape juice, soy milk, cranberry juice, and orange juice were described as drivers of acute food exposure.

Table 35. Acute Dietary Exposure for CPF

Population Subgroup	Oral aPoD (mg/kg)[a]	Residues at 99.9th Percentile Exposure (mg/kg/d)
All Infants < 1 year old	0.600	0.000273
Children 1-2 years old	0.581	0.000423
Children 6-12 years old	0.530	0.000189
Females 13-49 years old	0.469	0.000150

[a] aPoD = acute point of departure; Reference: US EPA (2014a)

Table 36. Steady-State Dietary Exposure for CPF

Population Subgroup	Oral ssPoD (mg/kg)[a]	Residues at 99.9th Percentile Max Exposure (mg/kg/d)
All Infants < 1 year old	0.103	0.000186
Children 1-2 years old	0.099	0.000242
Children 6-12 years old	0.090	0.000128
Females 13-49 years old	0.078	0.000075

[a] ssPoD = Steady State point of departure
Reference: US EPA (2014a)

IV.B.5. HHA Drinking Water Assessment

IV.B.5.a. Summary of US EPA Drinking Water Assessments

US EPA conducted a preliminary drinking water assessment (DWA) in 2011 and updated it with additional analyses in 2014 (US EPA, 2011a; US EPA, 2014c). CPF is rapidly oxidized to the oxon during the chlorination process of drinking-water treatment. Since more than 75% of community water systems in the US use chlorination to disinfect drinking water, the DWA assumed that CPF is converted 100% to CPF-oxon during water treatment processes. A drinking water level of concern (DWLOC) of 3.9 ppb was calculated for exposure to CPF-oxon based on the ssPoD, uncertainty factors, and estimated food exposure for infants.

Several use scenarios were expected to result in surface water concentrations that exceed the DWLOC, based on computer modeling. Concentrations in ground water were not expected to exceed the DWLOC. The updated DWA examined water monitoring programs across the country, including DPR's program, and found that none (except a registrant study of Orestimba Creek in Stanislaus County) were capable of detecting peak or 21-day average concentrations of CPF or CPF-oxon because the frequency of monitoring did not coincide with either the exposure period of interest or the timing of CPF applications.

- **Drinking water derived from ground water (i.e., wells) is predicted[3] to have acceptable levels** of CPF and CPF-oxon. Even for a use scenario with 5 applications per year totaling 14.5 lbs CPF per acre, the 21-day average concentration of CPF-oxon in drinking water derived from ground water is not expected to be greater than 0.15 µg / L (US EPA, 2014c).

[3] **For drinking water derived from ground water, source of predictions for Estimated Drinking Water Concentrations (EDWC):** For drinking water derived from ground water, USEPA (2014c) used the higher prediction from either of two models: Screening Concentration in Ground water (SCI-GROW) version 2.3, and Pesticide Root Zone Model for Ground Water (PRZM-GM). A previous evaluation by US EPA showed that, "In a few cases PRZM-GM underestimated pesticide concentration observed in ground water", especially "pesticide concentrations with high sorption coefficients (i.e., K_{OC} > 1,000 mL/g_{OC}) and low persistence (i.e., soil half-life < 30 days)." Quote is from: http://www.epa.gov/oppefed1/models/water/przm_gw/wqtt_przm_gw_guidance.htm Chlorpyrifos and chlorpyrifos-oxon both have lower K_{OC} values and longer soil half-lives that fall outside of those problematic ranges.

That is less than 4% of the Drinking Water Level of Concern (DWLOC) of 3.9 µg / L for CPF-oxon[4].

- **Drinking water derived from surface water is predicted[5] to pose an exposure concern** (Table 37). According to US EPA, several CPF uses may exceed the DWLOC at rates lower than maximum labeled rates (both single as well as yearly), including an application rate of one pound per acre per year (US EPA, 2014c). Uses that may exceed the DWLOC include scenarios for certain California cropping systems, such as wheat, rangeland, cole crops, and wine grapes.

- **Exceedances in drinking water derived from surface water are predicted to be highly localized.** Highest exposures are predicted in small watersheds where there is a high percent cropped area on which CPF is applied. Similarly, evaluation of surface water monitoring data illustrates that exposures are highly localized. Overall, model predictions agree well with surface water monitoring data, despite limitations of monitoring[6].

- **Routine treatment of drinking water is not expected to mitigate the risk.** According to US EPA, drinking water treatment processes in general are not efficient in removing pesticide residues. The exceptions may be granular activated carbon filtration or water softening, which may alter the water pH or provide a substrate for binding or deposition (US

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[4] **Calculation of Drinking Water Level of Comparison (DWLOC):** The average 21-day concentration of chlorpyrifos-oxon necessary to cause 10% AChE inhibition was determined by the US EPA Office of Pesticide Programs Health Effects Division to be 217 ppb. This value was divided by the safety factors (50x), resulting in a value of 4.3 ppb; and then the contribution from food (0.4 ppb) was subtracted out to give a DWLOC of 3.9 ppb. Source: USEPA 2014c, page 4, footnote 12. Though never stated by US EPA (2014c), the value 217 ppb corresponds to infants, the most susceptible population; see US EPA 2014a chlorpyrifos risk assessment Table 4.8.4. The 50x "safety factors" used by Bohaty (US EPA 2014a) comprises a 10x uncertainty factor as required by Food Quality Protection Act (FQPA) multiplied by a 5x uncertainty factor for intraspecific extrapolation. The intraspecific value is 5x for most populations, including infants; but for adult females, the intraspecific factor is 10x. Source: US EPA 2014a,b .

[5] **For drinking water derived from surface water, source of predictions for Estimated Drinking Water Concentrations (EDWC):** "Tier II surface water EDWCs for chlorpyrifos and chlorpyrifos-oxon were calculated using the Surface Water Concentration Calculator (SWCC) version 1.106. The SWCC uses Pesticide Root Zone Model for Ground Water version 5.0+ (PRZM5) and the Variable Volume Water Body Model (VVWM). PRZM5 is used to simulate pesticide transport as a result of runoff and erosion from an agricultural field. VVWM estimates environmental fate and transport of pesticides in surface water. The input parameters used in SWCC simulations are presented in Table 10" US EPA (2014c)

[6] **Limitations of surface-water monitoring to date:** " None of the monitoring programs examined to date were specifically designed to target chlorpyrifos use (except the Registrant Monitoring Program MRID 44711601); therefore, peak concentrations (and likely 21-day average concentrations) of chlorpyrifos and chlorpyrifos-oxon likely went undetected in these programs. In general, sampling frequency needs to be approximately equal to the duration of exposure concern. The chlorpyrifos monitoring data evaluated thus far also show that as sample frequency increases, so does the detection frequency" US EPA. 2014c. Chlorpyrifos: Updated Drinking Water Assessment for Registration Review, December 23, 2014. PC Code: 059101. DP Barcode: D424487, pp. 7-8).

EPA 2014c). Additionally, all CPF that enters a drinking water treatment facility is assumed to be converted to CPF-oxon during chlorination. And while CPF-oxon has a hydrolysis half-life of 5 days, the drinking water treatment simulation half-life for CPF-oxon is approximately 12 days. Therefore, once CPF-oxon forms during treatment, little transformation is expected to occur before consumption (during drinking water distribution) (US EPA 2014c).

IV.B.5.b. Risk Assessment Section (RAS) Evaluation of the Exposure to CPF in Drinking Water in California

In the absence of modeling data specific for California, the assessment utilized residue data from PDP's drinking water study and from the testing of surface and ground water in California to evaluate the potential exposure to CPF through drinking water.

IV.B.5.c. Analysis of Drinking Water Exposure Using PDP Residue Data

The PDP Drinking Water Project began in 2001 and ended in 2013 (PDP, 2015). The data include samples collected from water treatment plants located in agricultural areas, paired pre-treatment and post-treatment samples from water treatment plants, bottled water, and potable ground water. A total of 1835 samples were analyzed for CPF and/or CPF-oxon and no residues were detected. LODs ranged from 3 to 30 ppt for CPF and 12 to 510 ppt for CPF-oxon (Table 37). The average LOD for CPF-oxon in finished (treated) water samples (n = 706) was 38.2 ppt. Exposure to CPF-oxon in drinking water was estimated by assuming that each of the 706 samples of finished (treated) water contained CPF-oxon at concentrations equivalent to the LOD for CPF-oxon in each sample. The 95th and 99.9th percentile exposures for all infants, the most highly exposed subpopulation, were 0.000004 and 0.000108 mg/kg respectively (Table 38).

Table 37. PDF Monitoring Data for CPF and CPF-oxon in Ground Water, Untreated Drinking Water, Finished Drinking Water, and Bottled Water in California (2001-2013)

YEAR	CHEMICAL	SAMPLE TYPE	SAMPLES	DETECTS	LOD (PPT)
2001	CPF	Finished	134	0	11
	CPF-oxon	Finished	134	0	20
2002	CPF	Finished	267	0	6
	CPF-oxon	Finished	265	0	12
2003	CPF	Finished	272	0	9
	CPF-oxon	Finished	272	0	12
2004	-- NO DATA --				
2005	CPF	Bottled	93	0	30
	CPF	Finished	26	0	11
	CPF	Untreated	28	0	11
	CPF-oxon	Finished	26	0	510
	CPF-OXON	Untreated	28	0	510
2006	CPF	Bottled	88	0	30
	CPF	Finished	9	0	11

YEAR	CHEMICAL	SAMPLE TYPE	SAMPLES	DETECTS	LOD (PPT)
	CPF	Untreated	9	0	11
	CPF-oxon	Finished	9	0	510
	CPF-oxon	Untreated	9	0	510
2007	CPF	Ground water	4	0	30
2008	CPF	Ground water	2	0	30
2009	CPF	Ground water	13	0	30
2010	CPF	Ground water	27	0	30
2012	CPF	Untreated	26	0	30
	CPF	Finished	26	0	30
	CPF-oxon	Untreated	26	0	12
	CPF-oxon	Finished	26	0	12
2013	CPF	Ground water	8	0	30
	CPF-oxon	Ground water	8	0	12

LOD = limit of detection.

Table 38. DEEM-FCID (v. 3.18) Acute Exposure Estimates for CPF-Oxon in Drinking Water Based on 2001-2013 PDP Residue Data for CPF-Oxon in Treated (Finished) Water

Probabilistic Estimate With All Non-Detects at the LOD[a]			
Population Subgroup	Exposure (mg/kg/d)[b]		
	95th Percentile (Users)	99th Percentile (Users)	99.9th Percentile (Users)
All Infants (< 1 year old)	0.000004	0.000061	0.000108
Children 1-2 years old	0.000002	0.000025	0.000057
Children 6-12 years old	0.000002	0.000015	0.000036
Females 13-49 years old	0.000001	0.000017	0.000036

a- Residue data were assigned to commodities: "Water, direct, all sources", "Water, indirect, all sources".

b- 706 samples, no detections. LODs ranged 12-510 ppt (mean = 38.2 ppt).

IV.B.5.d. Analysis of Drinking Water Exposure Using EMON Surface Water Residue Data

DPR's Environmental Monitoring Branch collects residue data from surface water samples within California by a number of government agencies including the US Geologic Survey, the State Water Resources Control Board, and CALFED Bay-Delta Program, as well DPR sampling. The samples may be collected from water sources that are ultimately treated and used for drinking water as well as from irrigation ponds, sloughs, and agricultural drains that are either not used for drinking water or are located far from water bodies that may ultimately be used for drinking water, and therefore highly diluted before use. A total of 7154 samples of California surface water were analyzed for CPF from 2005 to 2014 and the range of detected residues was 0.000572 to 3.7 ppb. A total of 794 samples were analyzed for CPF-oxon and there were no detected residues (average detection limit ranged from 0.05 to 0.08 ppb) (Table 39) (CDPR, 2015a).

Exposure to CPF-oxon in drinking water was estimated by conducting a probabilistic analysis using either the detected CPF residue in surface water or the detection limit (in the case of non-detects) together with all individual water consumption records for each subpopulation. The DEEM-FCID residue data file (RDF) contained 7048 residue values (either the measured residue or LOD). The 95th and 99.9th percentile exposures for all infants, the most highly exposed subpopulation, were 0.000008 and 0.000419 mg/kg, respectively (Table 40). These exposures were up to 4-fold higher than the exposures estimated based on the PDP monitoring data.

Table 39. Summary of DPR Surface Water Monitoring for CPF in California (2005-2014)

YEAR	CHEMICAL	SAMPLE COUNT	DETECTS	DETECTION FREQUENCY (%)	RANGE (PPB)	AVG. AVG. DETECTION LIMIT FOR NON-DETECTS (PPB)
2005	CPF	702	59	8.4%	0.0058 - 1.4	0.0619
	CPF-oxon	14	0	0.0%	n/a	0.0562
2006	CPF	545	57	10.5%	0.0092 - 0.72	0.0728
	CPF-oxon	45	0	0.0%	n/a	0.0562
2007	CPF	804	82	10.2%	0.0079 - 3.7	0.0280
	CPF-oxon	59	0	0.0%	n/a	0.0562
2008	CPF	965	146	15.1%	0.0010 - 1.8	0.0232
	CPF-oxon	71	0	0.0%	n/a	0.0548
2009	CPF	628	79	12.6%	0.000572 - 2.377	0.0266
	CPF-oxon	66	0	0.0%	n/a	0.0500
2010	CPF	857	138	16.1%	0.00248 - 1.988	0.0211
	CPF-oxon	57	0	0.0%	n/a	0.0519
2011	CPF	985	122	12.4%	0.0022 - 1.4	0.0129
	CPF-oxon	60	0	0.0%	n/a	0.0650
2012	CPF	393	66	16.8%	0.0027 - 0.2940	0.0640
	CPF-oxon	52	0	0.0%	n/a	0.0800
2013	CPF	905	60	6.6%	0.0024 - 1.59	0.0925
	CPF-oxon	0	n/a	n/a	n/a	n/a
2014	CPF	370	51	13.8%	0.0027 - 1.75	0.0853
	CPF-oxon	0	n/a	n/a	n/a	n/a

CPF = chlorpyrifos, CPF-oxon = chlorpyrifos-oxon

Table 40. DEEM-FCID (v. 3.18) Acute Exposure Estimates for CPF-oxon in Drinking Water Based on 2005-2014 Surface Water Residue Data

Probabilistic Estimate With All Non-Detects at the Detection Limit[a,b]			
Population Subgroup	Exposure (mg/kg/d)[c]		
	95th Percentile (Users)	99th Percentile (Users)	99.9th Percentile (Users)
All Infants (< 1 year old)	0.000008	0.000049	0.000419
Children 1-2 years old	0.000004	0.000023	0.000177
Children 6-12 years old	0.000002	0.000014	0.000110
Females 13-49 years old	0.000002	0.000015	0.000119

a- Residue data were assigned to commodities: "Water, direct, all sources", "Water, indirect, all sources".
b- 7048 samples, 860 detections (range, 0.000572-3.7; mean 0.125 ppb). LODs ranged 0.001-4 ppb, mean 0.045 ppb).
c- CPF exposure values were converted to CPF-oxon by applying a molecular weight correction factor (0.9541).

IV.B.6. Analysis of Drinking Water Exposure Using DPR Ground Water Residue Data

The Environmental Monitoring Branch of DPR collects residue data from sampling of ground water within California by a number of government agencies including US Geological Survey, CA State Water Resources Control Board, CA Department of Water Resources, CA Department of Public Health, as well as sampling by DPR. The samples are collected from a variety of wells including municipal, community, domestic and irrigation. A total of 2055 samples were analyzed for CPF from 2004 to 2013 and only two samples had detectible residues (in 2006, 0.006 and 0.008 ppb). The average detection limit for non-detects ranged from 0.005 to 1 ppb each year. A total of 1903 samples were analyzed for CPF-oxon on and there were no detected residues (average detection limit ranged from 0.05 to 0.06 ppb) (Table 41) (CDPR, 2015b).

Exposure to CPF-oxon in drinking water was estimated by conducting a probabilistic analysis using either the detected CPF residue in ground water or the detection limit (in the case of non-detects) together with all individual water consumption records for each subpopulation. The DEEM-FCID residue data file (RDF) contained 2055 residue values (either the measured residue or detection limit). The 95th and 99.9th percentile exposures for all infants, the most highly exposed subpopulation, were 0.000018 and 0.000222 mg/kg, respectively (Table 42).

Table 41. Summary of Ground Water Monitoring for CPF in California, 2004-2013

YEAR	CHEMICAL	SAMPLE COUNT	DETECTS	DETECTION FREQUENCY (%)	RANGE (PPB)	AVG. DETECTION LIMIT FOR NON-DETECTS (PPB)
2004	CPF	152	0	0.0%	n/a	0.0181
	CPF-oxon	151	0	0.0%	n/a	0.0560
2005	CPF	388	0	0.0%	n/a	0.0050
	CPF-oxon	388	0	0.0%	n/a	0.0560
2006	CPF	478	2	0.0%	0.006 - 0.008	0.0071
	CPF-oxon	477	0	0.0%	n/a	0.0560
2007	CPF	354	0	0.0%	n/a	0.0107
	CPF-oxon	352	0	0.0%	n/a	0.0560
2008	CPF	437	0	0.0%	n/a	0.0921
	CPF-oxon	395	0	0.0%	n/a	0.0553
2009	CPF	94	0	0.0%	n/a	0.0837
	CPF-oxon	78	0	0.0%	n/a	0.0500
2010	CPF	65	0	0.0%	n/a	0.0862
	CPF-oxon	60	0	0.0%	n/a	0.0500
2011	CPF	46	0	0.0%	n/a	0.9393
	CPF-oxon	2	0	0.0%	n/a	0.0600
2012	CPF	22	0	0.0%	n/a	1.0000
	CPF-oxon	0	n/a	n/a	n/a	n/a
2013	CPF	25	0	0.0%	n/a	1.0000
	CPF-oxon	0	n/a	n/a	n/a	n/a

CPF = chlorpyrifos, CPF-oxon = chlorpyrifos-oxon

Table 42. DEEM-FCID Acute Exposure Estimates for CPF-Oxon in Drinking Water Based on 2004-2013 Ground Water Residue Data

Probabilistic Estimate With All Non-Detects at the Detection Limit[a,b]			
Population Subgroup	**Exposure (mg/kg/d)[c]**		
	95th Percentile (Users)	**99th Percentile (Users)**	**99.9th Percentile (Users)**
All Infants (< 1 year old)	0.000018	0.000127	0.000222
Children 1-2 years old	0.000012	0.000054	0.000115
Children 6-12 years old	0.000008	0.000031	0.000075
Females 13-49 years old	0.000009	0.000036	0.000073

a- Residue data were assigned to commodities: "Water, direct, all sources", "Water, indirect, all sources".
b- 2055 samples, 2 detects (0.006, 0.008 ppb). Detection limit for non-detects ranged 0.004-1 ppb (mean 0.072 ppb).
c- CPF exposure values were converted to CPF-oxon by applying a molecular weight correction factor (0.9541).

V. RISK CHARACTERIZATION

The critical NOELs or toxicological points of departure (PoDs) for characterizing the risk from exposures to CPF were PBPK-PD-estimated human equivalent doses. Risks were calculated as margins of exposure (MOE), a quotient of the NOEL and the human exposure level. A MOE of 100 was considered prudent for protection against the CPF toxicity. The target of 100 includes an uncertainty factor of 1 for interspecies sensitivity, an uncertainty factor of 10 for intraspecies variability, and an UF of 10 fold for potential neurodevelopmental effects.

V.A. Risk Characterization (Margins of Exposure) for a Single Route (oral, dermal, inhalation)

In the assessment of single routes of exposure, the risk for non-oncogenic effects is characterized in terms of a margin of exposure (MOE), defined as the ratio of the critical human equivalent PoD to the estimated human exposure levels. The calculation is shown below:

$$\text{Single Route MOE} = \frac{\text{PoD (e.g., oral, dermal, inhalation)}}{\text{Exposure Dosage (route specific: oral, dermal, inhalation)}}$$

V.B. Spray-Drift Bystander (Non-Occupational/Residential) Risk Characterization

Using the allowable application rates and methods specified on the product labels of currently registered CPF-containing products in California, the risk estimates (i.e., MOE) of different exposure routes associated with spray drift were evaluated: exposures through dermal contact and inhalation for females 13-49 years old and children 1-2 years old and exposures due to different mouthing activities associated with the small children (hand-to-mouth, object-to-mouth, and incidental soil ingestion). Because different portal-of-entries (dermal, inhalation, and oral) are involved, route-specific MOEs are used to characterize the risks associated with different exposure routes.

For females 13-49 years old, under the current buffer zone requirement of 25 feet, risks were estimated, for exposures associated with aerial applications via fixed-winged and rotor-wing aircraft at rates of 1, 2, or 2.3 lb AI/acre (Table 43) or ground boom and airblast at application rates of 1, 2, 4, or 6 lb AI/acre (Table 44). For aerial applications, aggregate risk at 10 ft for the Bell 205 helicopter scenario at 2 and 2.3 lb/ac application rates showed MOEs below 100. Inhalation and aggregated MOEs were less than 100 for all the 6 lb/acre ground boom and airblast applications at 25ft and 50 ft distances. The airblast 4 lb/acre aggregate MOEs were less than 100 at 25 ft.

For children 1-2 years old, risk estimates are of concern for exposures from inhalation routes at the lowest application rate of 1 lb AI/acre at 50 feet away from the edge of a treated field via aerial application (Table 45). When inhalation, dermal, and oral exposures associated with aerial applications are aggregated for children, risks of concern occur as far as 250 feet from the application. For dermal and oral exposures, no risks of concern were identified for children as close as 25 feet downwind of a ground boom application (Table 46), even at the highest allowed rate of 6 lb AI/acre. For inhalation and aggregate risk associated with ground boom applications, the MOEs were below 100 at 75 ft for 1 lb/ac, at 200 ft for 2 lb/ac aggregate, and 250 ft for 4 lb/ac to 6 lb/aggregate (Table 47). A risk of concern occurs for 1-2 year-old children 75 feet downwind of an airblast application at the rate of 6 lb AI/acre due to hand-to-mouth exposure (Table 48). Airblast inhalation and aggregate risk both show MOEs less than 100 at 75 ft for 1 lb/ac. Airblast inhalation MOEs were less than 100 at 200 ft for 2 lb/ac and 250 ft for 4 lb/ac and 6 lb/ac. Airblast Aggregate MOEs were below 100 at 75 ft for 1 lb/ac and 250 ft for 2 lb/ac, 4 lb/ac, and 6 lb/ac.

Table 43. MOEs for Females (13-49 years old) Associated with Spray Drift at Various Distances from a Field Treated with CPF Using Aerial Equipment

Scenarios	Spray Vol (gallon/acre)	Exposure Route	Appl. Rate	MOE at Various Distance Downwind from the Treated Fields						
			(lb/acre)	10 (feet)	25 (feet)	50 (feet)	100 (feet)	250 (feet)	500 (feet)	1000 (feet)
AT802A	2	Dermal	1	976	1144	1454	2158	4139	6945	13021
			2	486	572	729	1091	2180	4006	10190
			2.3	423	497	635	952	1905	3591	9264
		Inhalation	1	263	282	317	377	521	724	1309
			2	154	168	192	237	353	554	1183
			2.3	144	156	180	223	336	533	1139
		Aggregated MOE (Dermal & Inhalation Routes)	1	207	226	260	321	463	655	1189
			2	117	130	152	195	304	487	1060
			2.3	107	119	140	181	285	464	1014
Bell 205	2	Dermal	1	764	1207	1972	3244	5081	8562	17524
			2	379	596	968	1555	2807	5483	12500
			2.3	330	518	840	1347	2485	4941	11482
		Inhalation	1	214	256	312	389	554	831	1464
			2	123	152	191	250	399	661	1255
			2.3	114	141	179	236	385	641	1237
		Aggregated MOE (Dermal & Inhalation Routes)	1	167	211	270	348	500	758	1351
			2	93	121	160	215	350	590	1141
			2.3	85	111	147	201	333	567	1117

Table 44. MOEs for Females (13-49 years old) Associated with Spray Drift at Various Distances from a Field Treated with CPF Using Ground-based Equipment Ground Boom and Airblast

Scenarios	Swaths (percentile)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Ground boom										
High boom	40 (50^{th})	Dermal	1	19737	29762	39894	50676	69446	89287	110296
			2	9869	14881	19947	25338	34723	44644	55148
			4	4934	7441	9974	12669	17361	22322	27574
			6	3290	4960	6649	8446	11574	14881	18383
		Inhalation	1	282	317	349	377	429	477	521
			2	168	192	216	237	278	316	353
			4	103	122	140	158	193	228	267
			6	79	96	112	128	163	202	243
		Aggregated MOE (Dermal & Inhalation Routes)	1	278	314	346	375	427	474	519
			2	165	190	213	235	276	314	351
			4	101	120	138	156	191	226	265
			6	77	94	110	126	161	199	240
High boom	40 (90^{th})	Dermal	1	13889	19330	25000	31250	41667	52084	62501
			2	6945	9665	12500	15625	20834	26042	31250
			4	3472	4833	6250	7813	10417	13021	15625
			6	2315	3222	4167	5208	6945	8681	10417
		Inhalation	1	282	317	349	377	429	477	521
			2	168	192	216	237	278	316	353
			4	103	122	140	158	193	228	267
			6	79	96	112	128	163	202	243
		Aggregated MOE (Dermal & Inhalation Routes)	1	276	312	344	373	425	472	517
			2	164	188	212	234	274	312	349
			4	100	119	137	155	189	224	263
			6	76	93	109	125	159	197	238
Low boom	40 (50^{th})	Dermal	1	37501	55148	72117	93751	125002	156252	187503
			2	18750	27574	36058	46876	62501	78126	93751
			4	9375	13787	18029	23438	31250	39063	46876
			6	6250	9191	12019	15625	20834	26042	31250
		Inhalation	1	282	317	349	377	429	477	521
			2	168	192	216	237	278	316	353
			4	103	122	140	158	193	228	267
			6	79	96	112	128	163	202	243
		Aggregated MOE (Dermal & Inhalation Routes)	1	280	315	347	376	428	475	520
			2	166	191	214	236	276	315	352
			4	102	121	139	157	192	227	266
			6	78	95	111	127	162	200	241
Low boom	40 (90^{th})	Dermal	1	22059	30242	39063	48078	64656	78126	93751
			2	11030	15121	19532	24039	32328	39063	46876
			4	5515	7561	9766	12019	16164	19532	23438
			6	3677	5040	6511	8013	10776	13021	15625
		Inhalation	1	282	317	349	377	429	477	521
			2	168	192	216	237	278	316	353
			4	103	122	140	158	193	228	267
			6	79	96	112	128	163	202	243
		Aggregated MOE (Dermal & Inhalation Routes)	1	279	314	346	374	426	474	518
			2	165	190	213	235	275	313	351
			4	101	120	138	156	191	226	264
			6	77	94	110	126	161	199	239

Scenarios	Swaths (percentile)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Airblast										
Dormant Apples	60	Dermal	1	3388	8903	18151	31943	75606	143132	237346
			2	1694	4452	9076	15971	37803	71566	118673
			4	847	2226	4538	7986	18902	35783	59336
			6	565	1484	3025	5324	12601	23855	39558
		Inhalation	1	282	317	349	377	430	477	521
			2	168	192	216	237	278	315	353
			4	103	122	140	158	193	229	267
			6	79	96	112	128	163	202	243
		Aggregated MOE (Dermal & Inhalation Routes)	1	260	306	343	373	428	475	520
			2	152	184	211	234	276	314	352
			4	92	116	136	155	191	227	266
			6	69	90	108	125	161	200	242
Sparse Orchard	60	Dermal	1	4178	9173	16333	25580	50269	83335	124174
			2	2089	4587	8167	12790	25134	41667	62087
			4	1044	2293	4083	6395	12567	20834	31044
			6	696	1529	2722	4263	8378	13889	20696
		Inhalation	1	282	317	349	377	430	477	521
			2	168	192	216	237	278	315	353
			4	103	122	140	158	193	229	267
			6	79	96	112	128	163	202	243
		Aggregated MOE (Dermal & Inhalation Routes)	1	264	306	342	372	426	474	519
			2	155	184	210	233	275	313	351
			4	94	116	135	154	190	226	265
			6	71	90	107	125	160	199	240

Table 45. MOEs for Children (1-2 years old) Associated with Spray Drift at Various Distances from a Field Treated with CPF Using Aerial Equipment

Scenarios	Spray Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				10 (feet)	25 (feet)	50 (feet)	100 (feet)	250 (feet)	500 (feet)	1000 (feet)
AT802A	2	Dermal	1	3786	4440	5641	8374	16063	26951	50532
			2	1886	2218	2829	4236	8461	15548	39547
			2.3	1640	1930	2464	3696	7392	13937	35952
		Object-to-Mouth	1	4460	5230	6645	9864	18922	31747	59526
			2	2222	2613	3333	4989	9967	18316	46585
			2.3	1932	2274	2903	4354	8708	16418	42350
		Hand-to-Mouth	1	137	161	204	303	581	975	1827
			2	68	80	102	153	306	562	1430
			2.3	59	70	89	134	267	504	1300
		Soil Ingestion	1	18347	21515	27335	40578	77842	130601	244877
			2	9140	10751	13710	20525	41003	75347	191643
			2.3	7948	9354	11940	17911	35821	67539	174221
		Inhalation	1	75	81	90	108	147	203	365
			2	43	48	54	68	100	155	329
			2.3	41	45	51	64	95	149	318
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	47	53	61	78	116	166	300
			2	26	29	35	46	74	120	264
			2.3	23	27	32	42	69	113	252
Bell 205	2	Dermal	1	2965	4686	7652	12589	19720	33227	68006

Scenarios	Spray Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				10 (feet)	25 (feet)	50 (feet)	100 (feet)	250 (feet)	500 (feet)	1000 (feet)
			2	1472	2312	3755	6034	10893	21277	48511
			2.3	1280	2009	3262	5229	9646	19174	44560
		Object-to-Mouth	1	3493	5519	9013	14830	23230	39140	80109
			2	1734	2723	4423	7108	12832	25063	57145
			2.3	1508	2366	3842	6160	11362	22587	52491
		Hand-to-Mouth	1	107	169	277	455	713	1202	2459
			2	53	84	136	218	394	769	1754
			2.3	46	73	118	189	349	693	1611
		Soil Ingestion	1	14369	22706	37079	61007	95562	161015	329554
			2	7135	11201	18195	29239	52788	103106	235082
			2.3	6202	9734	15806	25341	46742	92918	215936
		Inhalation	1	58	71	86	108	155	232	409
			2	33	41	52	69	110	182	349
			2.3	31	39	49	65	107	178	345
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	37	49	65	86	126	192	347
			2	20	27	37	51	85	145	287
			2.3	18	25	34	48	80	140	280

Table 46. MOEs for Children (1-2 years old) Associated with Spray Drift at Various Distances from a Field Treated with CPF Using Ground Boom

Scenarios	Swaths (Percentile)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
High Boom	40 (50^{th})	Dermal	1	76596	115503	154823	196667	269506	346508	428039
			2	38298	57751	77411	98333	134753	173254	214019
			4	19149	28876	38706	49167	67377	86627	107010
			6	12766	19250	25804	32778	44918	57751	71340
		Object-to-Mouth	1	90229	136059	182377	231668	317471	408177	504218
			2	45114	68029	91188	115834	158735	204088	252109
			4	22557	34015	45594	57917	79368	102044	126055
			6	15038	22676	30396	38611	52912	68029	84036
		Hand-to-Mouth	1	2770	4177	5599	7112	9746	12531	15479
			2	1385	2088	2799	3556	4873	6265	7739
			4	692	1044	1400	1778	2436	3133	3870
			6	462	696	933	1185	1624	2088	2580
		Soil Ingestion	1	371182	559719	750261	953035	1306011	1679156	2074252
			2	185591	279859	375131	476517	653005	839578	1037126
			4	92795	139930	187565	238259	326503	419789	518563
			6	61864	93286	125044	158839	217668	279859	345709
		Inhalation	1	81	90	99	108	122	135	147
			2	48	54	61	68	79	90	100
			4	30	34	40	45	55	65	75
			6	23	27	32	36	47	57	68
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	79	88	97	106	121	134	146
			2	46	53	60	66	78	88	99
			4	29	33	39	44	54	63	74
			6	22	26	30	35	45	56	66
High Boom	40 (90^{th})	Dermal	1	53901	75017	97022	121278	161704	202130	242555
			2	26951	37509	48511	60639	80852	101065	121278

Scenarios	Swaths (Percentile)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
			4	13475	18754	24256	30319	40426	50532	60639
			6	8984	12503	16170	20213	26951	33688	40426
		Object-to-Mouth	1	63494	88368	114289	142862	190482	238103	285724
			2	31747	44184	57145	71431	95241	119052	142862
			4	15874	22092	28572	35715	47621	59526	71431
			6	10582	14728	19048	23810	31747	39684	47621
		Hand-to-Mouth	1	1949	2713	3509	4386	5848	7309	8771
			2	975	1356	1754	2193	2924	3655	4386
			4	487	678	877	1096	1462	1827	2193
			6	325	452	585	731	975	1218	1462
		Soil Ingestion	1	261202	363529	470164	587705	783606	979508	1175410
			2	130601	181764	235082	293852	391803	489754	587705
			4	65301	90882	117541	146926	195902	244877	293852
			6	43534	60588	78361	97951	130601	163251	195902
		Inhalation	1	81	90	99	108	122	135	147
			2	48	54	61	68	79	90	100
			4	30	34	40	45	55	65	75
			6	23	27	32	36	47	57	68
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	78	87	96	105	120	133	145
			2	46	52	59	66	77	87	98
			4	28	33	38	43	53	62	73
			6	21	25	30	35	44	54	65

Table 47. MOEs for Children (1-2 years old) Associated with Spray Drift at Various Distances from a Field Treated with CPF Using Low Boom Ground Boom

Scenarios	Swaths (Percentile)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Low Boom	40 (50^{th})	Dermal	1	145533	214019	279872	363833	485111	606389	727666
			2	72767	107010	139936	181917	242555	303194	363833
			4	36383	53505	69968	90958	121278	151597	181917
			6	24256	35670	46645	60639	80852	101065	121278
		Object-to-Mouth	1	171434	252109	329681	428585	571447	714309	857171
			2	85717	126055	164841	214293	285724	357155	428585
			4	42859	63027	82420	107146	142862	178577	214293
			6	28572	42018	54947	71431	95241	119052	142862
		Hand-to-Mouth	1	5263	7739	10121	13157	17543	21928	26314
			2	2631	3870	5060	6579	8771	10964	13157
			4	1316	1935	2530	3289	4386	5482	6579
			6	877	1290	1687	2193	2924	3655	4386
		Soil Ingestion	1	705246	1037126	1356242	1763114	2350819	2938524	3526229
			2	352623	518563	678121	881557	1175410	1469262	1763114
			4	176311	259282	339060	440779	587705	734631	881557
			6	117541	172854	226040	293852	391803	489754	587705
		Inhalation	1	81	90	99	108	122	135	147
			2	48	54	61	68	79	90	100

Scenarios	Swaths (Percentile)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
			4	30	34	40	45	55	65	75
			6	23	27	32	36	47	57	68
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	80	89	98	107	122	134	146
			2	47	53	61	67	78	89	99
			4	29	34	39	44	54	64	74
			6	22	26	31	36	46	56	67
Low Boom	40 (90th)	Dermal	1	85608	117366	151597	186581	250919	303194	363833
			2	42804	58683	75799	93291	125460	151597	181917
			4	21402	29341	37899	46645	62730	75799	90958
			6	14268	19561	25266	31097	41820	50532	60639
		Object-to-Mouth	1	100844	138253	178577	219787	295576	357155	428585
			2	50422	69127	89289	109894	147788	178577	214293
			4	25211	34563	44644	54947	73894	89289	107146
			6	16807	23042	29763	36631	49263	59526	71431
		Hand-to-Mouth	1	3096	4244	5482	6747	9074	10964	13157
			2	1548	2122	2741	3374	4537	5482	6579
			4	774	1061	1371	1687	2268	2741	3289
			6	516	707	914	1125	1512	1827	2193
		Soil Ingestion	1	414850	568747	734631	904161	1215941	1469262	1763114
			2	207425	284373	367315	452081	607970	734631	881557
			4	103713	142187	183658	226040	303985	367315	440779
			6	69142	94791	122438	150694	202657	244877	293852
		Inhalation	1	81	90	99	108	122	135	147
			2	48	54	61	68	79	90	100
			4	30	34	40	45	55	65	75
			6	23	27	27	36	27	27	68
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	79	88	97	106	121	134	145
			2	47	53	60	66	78	88	98
			4	29	33	39	44	54	63	73
			6	22	26	30	35	45	55	66

Table 48. MOEs for Children (1-2 years old) Associated with Spray Drift at Various Distances from a Field Treated with CPF Using Airblast

Scenarios	Swaths	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Dormant Apples	60	Dermal	1	13147	34552	70442	123964	293414	555470	921096
			2	6573	17276	35221	61982	146707	277735	460548
			4	3287	8638	17611	30991	73353	138868	230274
			6	2191	5759	11740	20661	48902	92578	153516
		Object-to-Mouth	1	15486	40701	82979	146026	345633	654329	1085027
			2	7743	20351	41489	73013	172817	327164	542513
			4	3872	10175	20745	36506	86408	163582	271257
			6	2581	6784	13830	24338	57606	109055	180838
		Hand-to-	1	475	1249	2547	4483	10611	20087	33309

Scenarios	Swaths	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distance Downwind from the Treated Fields						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
		Mouth	2	238	625	1274	2241	5305	10044	16655
			4	119	312	637	1121	2653	5022	8327
			6	79	208	425	747	1768	3348	5552
		Soil Ingestion	1	63708	167437	341358	600720	1421866	2691777	4463580
			2	31854	83719	170679	300360	710933	1345889	2231790
			4	15927	41859	85340	150180	355467	672944	1115895
			6	10618	27906	56893	100120	236978	448630	743930
		Inhalation	1	81	90	99	108	122	135	147
			2	48	54	61	68	79	90	100
			4	30	34	40	45	55	65	75
			6	23	27	32	36	47	57	68
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	69	83	95	105	121	134	147
			2	39	50	58	66	78	89	99
			4	23	31	37	43	54	64	75
			6	17	24	29	35	45	56	67
Sparse Orchard	60	Dermal	1	16214	35600	63386	99272	195085	323407	481898
			2	8107	17800	31693	49636	97542	161704	240949
			4	4053	8900	15846	24818	48771	80852	120475
			6	2702	5933	10564	16545	32514	53901	80316
		Object-to-Mouth	1	19099	41936	74666	116940	229805	380965	567663
			2	9550	20968	37333	58470	114902	190482	283831
			4	4775	10484	18667	29235	57451	95241	141916
			6	3183	6989	12444	19490	38301	63494	94610
		Hand-to-Mouth	1	586	1287	2292	3590	7055	11695	17427
			2	293	644	1146	1795	3527	5848	8713
			4	147	322	573	897	1764	2924	4357
			6	98	215	382	598	1176	1949	2904
		Soil Ingestion	1	78570	172516	307163	481068	945370	1567213	2335251
			2	39285	86258	153581	240534	472685	783606	1167625
			4	19643	43129	76791	120267	236342	391803	583813
			6	13095	28753	51194	80178	157562	261202	389208
		Inhalation	1	81	90	99	108	122	135	147
			2	48	54	61	68	79	90	100
			4	30	34	40	45	55	65	75
			6	23	27	32	36	47	57	68
		Aggregated MOE (Dermal, Oral & Inhalation Routes)	1	71	84	95	104	120	134	146
			2	41	50	58	65	77	88	99
			4	24	31	37	43	53	63	74
			6	18	24	29	34	45	55	66

V.C. Comparison of Spray Drift Exposure Assessment modeling for CPF with US EPA

Both US EPA and HHA produced the CPF horizontal deposition estimates using computer simulation models. Inputs for some scenarios modeled were similar. For other scenarios, the

inputs were quite different. Details about the models, the modeling process, and estimates that this risk assessment produced can be found in Appendix 2 (Barry, 2017).

V.C.1. Orchard Airblast and Ground Boom

For orchard airblast and ground boom downwind deposition, this exposure assessment used AgDRIFT 2.0.05 because we did not have access to AgDRIFT 2.1.1 regulatory version before the analysis was completed. For orchard airblast and ground boom, AgDRIFT 2.0.05 yielded results identical to AgDRIFT 2.1.1 regulatory. This is expected because the empirical models that produce the orchard airblast and ground boom results have not changed since the earliest versions of AgDRIFT following the expert panel review in the mid-1990s.

V.C.1.a. Orchard Airblast

This exposure assessment includes inhalation exposures using surrogate air concentrations from AGDISP model runs for the AT802A aircraft with 2 GPA finished spray. US EPA did not include inhalation in the exposure assessment for orchard airblast. However, with respect to horizontal deposition, US EPA and this exposure assessment for orchard airblast are consistent. The only differences are due to US EPA rounding up to 2 decimal places for the horizontal deposition. US EPA presented only the sparse orchard scenario. This exposure assessment presented sparse orchard and dormant apples. A side-by-side comparison for sparse orchard and 2 lb ai/ac application rate is shown in Table 49.

Table 49. Comparison of 50th Percentile Sparse Orchard Horizontal Deposition (pounds per active ingredient per acre [lb AI/ac] Across a 50 ft Wide Lawn for 20 Swaths and 2 lb AI/ac Application Rate as Estimated Using the AgDRIFT Model

Distance Downwind (ft)	This Exposure Assessment	US EPA
0	*[a]	0.57[b]
10	*	0.16
25	0.0886	0.09
50	0.04	0.04
75	0.022	0.02
100	0.0136	0.01
125	0.009	0.01
150	0.0064	0.01
200	0.0036	0.00
250	0.0022	0.00
300	0.0016	0.00

a- This exposure assessment did not report estimates for empirical model fits between 0 and 25 feet because no field measurements were made within that distance range. The empirical model fit starts at 25 ft downwind of the treated field.

b- These horizontal deposition estimates are in error (References: Personal Communication with Charles Peck; (US EPA, 2014a)).

V.C.1.b. Ground Boom

This exposure assessment includes inhalation exposures using surrogate air concentrations from AGDISP model runs for the AT802A aircraft with 2 GPA finished spray. US EPA did not include inhalation in the exposure assessment for ground boom. With respect to the inputs for

horizontal deposition estimation, US EPA and this exposure assessment for ground boom are consistent. Both used the same AgDRIFT Fine to Medium/Coarse droplet spectra category for low and high boom applications. However, US EPA reported the 90th percentile estimates. This exposure assessment reported the 50th percentile estimates because the orchard airblast and aerial are both 50th percentile estimates. The use of the 50th percentile estimate puts ground boom on the same estimation basis as orchard airblast and aerial. Table 50 shows a side-by-side comparison of ground boom horizontal deposition (lb ai/ac) across a 50 ft wide lawn for 20 swaths and 2 lb ai/ac application rate as estimated using the AgDRIFT model.

Table 50. Comparison of Ground Boom Horizontal Deposition (lb AI/ac) across a 50ft Wide Lawn for 20 Swaths and 2 lb AI/ac Application Rate as Estimated Using the AgDRIFT Model

Distance Downwind (ft)	Low Boom[a] 50th Percentile	Low Boom 90th Percentile (US EPA)	High Boom[b] 50th Percentile	High Boom 90th Percentile (US EPA)
0	*[c]	0.46[d]	*	0.54[d]
10	*	0.02	*	0.04
25	0.0094	0.02	0.0184	0.03
50	0.0064	0.01	0.0118	0.02
75	0.0048	0.01	0.009	0.02
100	0.0040	0.01	0.0074	0.01
125	0.0034	0.01	0.0062	0.01
150	0.0030	0.01	0.0054	0.01
200	0.0024	0.00	0.0042	0.01
250	0.0020	0.00	0.0034	0.01
300	0.0018	0.00	0.0028	0.01

a- Low boom height is 20 inches above the target.
b- High boom is 50 inches above the target.
c-This exposure assessment did not report estimates for empirical model fits between 0 and 25 feet because no field measurements were made within that distance range. The empirical model fit starts at 25 ft downwind of the treated field.
d-These horizontal deposition estimates are in error (References: Personal Communication with Charles Peck; (US EPA, 2014a)).

V.C.2. Aerial Application

There are differences between US EPA and this exposure assessment for aerial modeling inputs. Thus, the horizontal deposition and air concentration estimates differ between US EPA and this exposure assessment. The most important difference is that this exposure assessment used AGDISP 8.28 (Teske and Curbishley, 2013) to simulate the aerial application scenarios while US EPA used AgDRIFT 2.1.1 regulatory version. The Tier I aerial default values are shown in the AgDRIFT user's manual (Teske et al., 2002b). For this comparison, the US EPA Tier II modeling inputs will be compared. Table 51 shows the input comparisons for the fixed wing aircraft scenario and follows the format of the tables shown in the AgDRIFT 2.0.05 user's manual (Teske et al., 2002b). The format of the AgDRIFT user's manual does not change with model version and the Tier I default parameter are the same between AgDRIFT 2.0.05 and AgDRIFT 2.2.1. AgDRIFT Tier I inputs are shown for the US EPA inputs, which were not changed by US EPA from the defaults.

Table 51. Details of Aerial Application Inputs for AgDRIFT and AGDISP used by US EPA and this Exposure Assessment

Parameters	**DPR AGDISP**	**US EPA AgDRIFT**
Aircraft Model	AT802A	AT401
Weight	11160 lbs	6000 lbs
Wing Semi-span	29 ft	24.5 ft
Flight Speed	144.99 mph	119.99 mph
Release Height	10 ft	10 ft
Number of Nozzles	39	42
Vertical Offset	-0.6601 ft	-1.51 ft
Horizontal Offset	-0.5 ft	-0.83 ft
Boom Span	76.3%	76.32%
Spacing (even)	14 inches	11 inches
ASABE[a] Droplet Spectra Classification	Medium	Tier I Fine to Medium Tier II Medium
Wind Speed at 2 m	10 mph	10 mph
Wind Direction	Perpendicular to Flight Path	Perpendicular to Flight Path
Surface Roughness	0.12 ft (low crops)	0.0246 ft (bare soil)
Stability	Overcast (Neutral)	Overcast (Neutral)
Relative Humidity	20%	50%
Temperature	90 deg F	86 deg F
Specific Gravity	1.0	1.0
Spray Volume Rate	2 gal/ac and 15 gal/ac	2 gal/ac
Application Rate	2 lb/ac[b]	2 lb/ac
Nonvolatile Rate	2 lb/ac	3 lb/ac[c]
Active Solution % of Tank Mix	12%	12%
Additive Solution % of Tank Mix	0%	5%
Nonvolatile Active	12%	12%
Volatile Fraction	0.88	0.83
Nonvolatile Fraction	0.12	0.17
Swath Width	60 ft	60 ft
Swath Displacement	37%	37%
Number of Flight Lines	50	20

a- American Society of Agricultural and Biological Engineers (formerly American Society of Agricultural Engineers [ASAE]); the organization changed its name in 2005.

b- Application rates of 1, 2, 2.3, 4, and 6 lb/ac were simulated at both 2 gal/ac and 15 gal/ac spray volumes. Although 4 and 6 lb/ac are not allowed for aerial application by the current product labels of CPF, these application rates were included in the US EPA analyses (Dawson et al., 2012). The employment of 15 gallons/acre for AGDISP simulation is to evaluate the effect of spray volume on the drift exposure estimates.

c- US EPA indicates in D3399483. Appendix F. CPOSDrift.xlsx: "…DAS Error Correction Comments/Meetings" for this tank mix but there is no accompanying documents to explain the "correction." Not all CPF products are manufactured by a single registrant and therefore, this exposure assessment does not include the 1 lb/ac of non-active ingredient-nonvolatile material in the tank mix. Available at https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0850-0107

Deposition estimates for 2 lb ai/ac application rate are compared in Table 52 and shown in Figure 12. US EPA AgDRIFT estimates were extended to 1000 ft downwind for comparison to DPR AGDISP estimates. In addition, the US EPA AgDRIFT inputs were used in AGDISP to provide a

comparison of AgDRIFT and AGDISP horizontal deposition estimate for the AT401 aircraft. The AgDRIFT 2.1.1 aerial algorithm does not include an evaporation time-step refinement that was incorporated into AGDISP 8.28 to improve mass accountancy (Per. Comm. Harold Thistle, 2014). AgDRIFT horizontal deposition is higher than AGDISP for the same scenario (AT401 aircraft) due to the lack of the refined evaporation time-step. Thus, for the same inputs, the AgDRIFT model will produce higher horizontal deposition estimates than AGDISP. For the same model (e.g., AGDISP), the horizontal deposition estimates of this exposure assessment are also higher than US EPA for several additional reasons: 1) the AT802A was selected as the California aircraft based on common use in California and higher horizontal deposition estimates, 2) this exposure assessment used 50 swathes to reflect the largest application sizes in California, 3) the meteorological conditions used in this exposure assessment are California specific, and 4) the tank mix fractions are generic. In addition, US EPA used simple multiplication of a base application rate AgDRIFT run to obtain deposition estimates for a variety of application rates. Analysis shown in Barry (2015) indicates that simple multiplication of the horizontal deposition fraction from a base application rate to adjust for desired application rates will not yield the same results as if the AGDISP model is run for each of the desired application rates (Figure 12). The difference is small in the near-field, but increases in the far field. Because of this effect, this exposure assessment did not use the simple multiplication method for the application rate adjustments. Instead, each application rate scenario was simulated. There is also a nonlinear effect of spray volume (gal/ac) on deposition at the same application rate, as illustrated by the effect of a spray volume of 2 gal/ac versus a spray volume of 15 gal/ac on horizontal deposition. As with application rate, the effect is largest in the far field (greater than 300 ft). This exposure assessment included the spray volume analysis as part of the higher application rates scenarios. However, spray volume has an effect at all application rates (Barry, 2017). The AT802A aircraft was used for these simulations. The simulation inputs are shown in Appendix 2.

Table 52. Comparison of Aerial Horizontal Deposition (Fraction of Application Rate) Aross a 50 ft Wide Lawn for 2 lb AI/ac Application Rate as Estimated Using the AgDRIFT and AGDISP Models

Downwind Distance (ft)	US EPA AgDRIFT 2 gal/ac 20 swath AT401 Tier I	US EPA AgDRIFT 2 gal/ac 20 swath AT401 Tier II	US EPA AGDISP 2 gal/ac 20 swath AT401	DPR AGDISP 2 gal/ac 50 swath AT802A	DPR AGDISP 15 gal/ac 50 swath AT802A
10	0.20	0.1800	0.1374	0.1929	0.1859
25	0.17	0.1500	0.1170	0.1640	0.1580
50	0.13	0.1100	0.0914	0.1286	0.1240
75	0.10	0.0800	0.0742	0.1034	0.0955
100	0.08	0.0700	0.0627	0.0859	0.0833
125	0.06	0.0500	0.0546	0.0739	0.0717
150	0.05	0.0500	0.0483	0.0652	0.0634
200	0.04	0.0400	0.0394	0.0524	0.0515
250	0.03	0.0300	0.0327	0.0430	0.0435
300	0.03	0.0300	0.0275	0.0365	0.0387
500	0.02	0.0154	0.0155	0.0234	0.0286
1000	*[1]	0.0048	0.0054	0.0092	0.0203

[1]AgDRIFT Tier I does not estimate to 1000 ft.

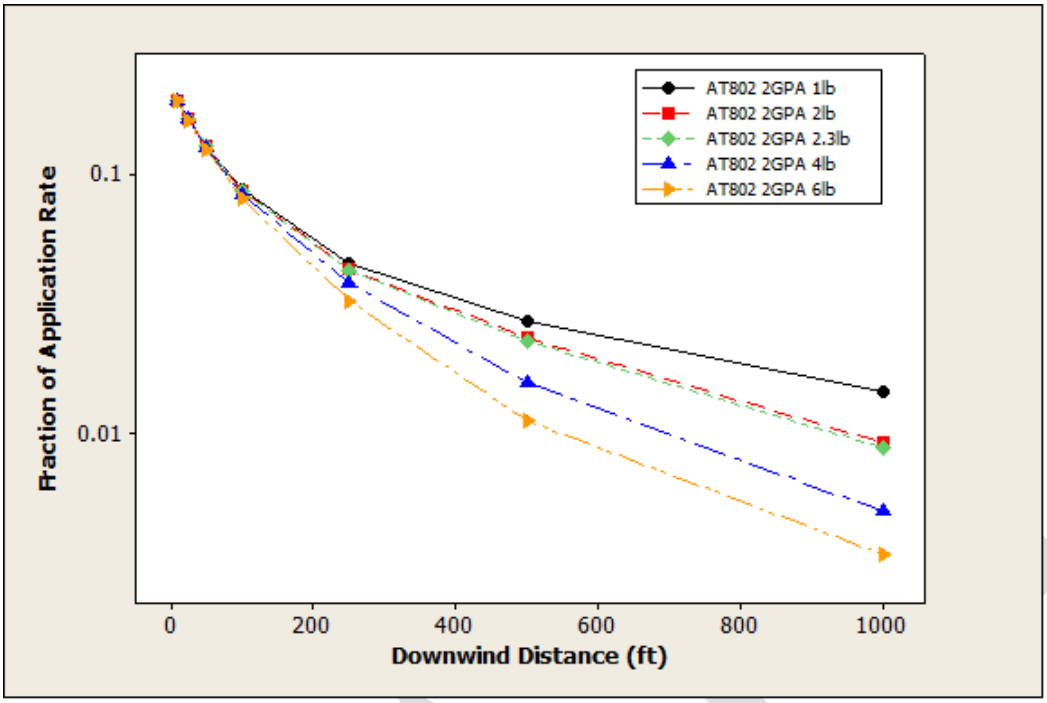

Figure 12. Effect of Application Rate on Aerial Application Downwind Horizontal Deposition Expresses as a Fraction of Application Rate

V.D. House Dust Risk Characterization

The short-term absorbed daily dose of chlorpyrifos via house dust is estimated to be 0.048 µg/kg/day in infant (i.e., <1 yr old). Comparing the estimated dose to an acute oral PoD (steady state) of 103 µg/kg/day for infants (US EPA, 2014a), the MOE of chlorpyrifos exposure due to house dust is 2146. Based on the results presented, chlorpyrifos exposure from house dust would not constitute more than 10% AChE inhibition in infants.

V.E. Dietary Risk Characterization

Dietary risk is characterized by the MOEs (calculation shown below) based on acute and steady-state PoDs for dietary CPF residues in the sensitive population subgroups (all infants <1 year old; children 1-2 years old, children 6-12 years old, and females 13-49 years old). The PoDs, residues, and MOEs for each population subgroup is shown below in Table 51.

V.E.1. Acute and Steady State Dietary (food only) Margins of Exposure

It is evident that using the PoDs from the PBPK-PD model for acute and steady-state oral (dietary: food only) exposures show that MOEs for CPF are all acceptable (Table 53). The MOEs were determined by using the oral acute PoD (aPoD) or the steady-state PoD (ssPoD) for each population subgroup and dividing it by the respective dietary exposures (MOE = aPoD or ssPoD ÷ exposure).

Table 53. Acute and Steady-state Dietary (food only) Exposure and Margins of Exposure for CPF

ACUTE DIETARY EXPOSURE[a]							
Population Subgroup	**aPoD[b, c] (mg/kg)**	**95th Percentile**		**99th Percentile**		**99.9th Percentile**	
		Exposure (mg/kg/d)	**MOE[d]**	**Exposure (mg/kg/d)**	**MOE[d]**	**Exposure (mg/kg/d)**	**MOE[d]**
All Infants:< 1 yr	0.600	0.000050	12,000	0.000088	6,818	0.000273	2,198
Children: 1-2 yrs	0.581	0.000082	7,085	0.000143	4,063	0.000423	1,374
Children: 6-12 yrs	0.530	0.000040	13,250	0.000072	7,361	0.000189	2,804
Females: 13-49 yrs	0.469	0.000021	22,333	0.000041	11,439	0.000150	3,127
STEADY STATE (21-DAY) DIETARY EXPOSURE[a]							
Population Subgroup	**ssPoD[b, e] (mg/kg)**	**70th Percentile**		**95th Percentile**		**99.9th Percentile**	
		Max. Exposure (mg/kg)	**MOE[d]**	**Exposure (mg/kg/d)**	**MOE[d]**	**Exposure (mg/kg/d)**	**MOE[d]**
All Infants:< 1 yr	0.103	0.000020	5,150	0.000045	2,289	0.000186	554
Children: 1-2 yrs	0.099	0.000038	2,605	0.000072	1,375	0.000242	409
Children: 6-12 yrs	0.090	0.000019	4,737	0.000039	2,308	0.000128	703
Females: 13-49 yrs	0.078	0.000009	8,667	0.000018	4,333	0.000075	1,040

a- Exposures are from the US EPA dietary exposure assessment to support registration review (US EPA, 2014b)
b- Point of Departures are PBPK-PD-estimated human equivalent doses
c- aPoD = acute point of departure
d- Margin of Exposure (MOE) = PoD ÷ Dietary Exposure. Target MOE is 100 for every population.
e- ssPoD = steady-state (21 day) point of departure

V.E.2. Drinking Water Exposure

V.E.2.a. Acute Drinking Water Margins of Exposure

It was necessary to perform a conversion from CPF to CPF-oxon values. Acute CPF PoDs from PBPK-PD modeling of dietary (food only) exposures were selected since they were the highest and because exposure to dietary residues is usually one event rather than continuous. As shown in Table 54, the CPF-oxon (ppb), water concentration (L) and body weights obtained from the US EPA 2014 Revised Human Health Risk Assessment were used to calculate the CPF-oxon PoD (µg/kg/d) (e.g., [CPF-oxon PoD (ppb) x water concentration (L)] ÷ body weight (kg) = CPF-oxon PoD µg/kg/d) (US EPA, 2014a). The ratio (Total Equivalent Residue: TEF) of CPF-oxon µg/kg/d to CPF µg/kg/d PoD yielded similar values among all population subgroups. Infants (<1 year old) and children (1-2 years old) had similar PoDs for CPF-oxon and similar TEFs (Table 54). The MOEs were calculated as follows: MOE_{DW} = (CPF-oxon PoD ÷ $DW_{PDP\ or\ EMON}$ Residue). DW MOEs indicate that there is no risk from drinking water exposure in California based on both PDP and EMON data.

Table 54. Acute CPF to CPF-Oxon Conversion for Drinking Water Residue Assessment

Population Subgroup	CPF-oxon PoD (ppb)	Water Cons. (L)	Body Weight (kg)[a]	CPF-Oxon PoD mg/kg/d	CPF PoD mg/kg/d	TEF[b]
Infants < 1 yr	1,183	0.688	4.8	0.170	0.600	3.53
Children 1-2 yrs	3,004	0.688	13	0.159	0.581	3.65
Children 6-12 yrs	7,700	0.688	37.1	0.143	0.530	3.71
Youth 13-19 yrs	4,988	1.71	67.31	0.127	0.475	3.74
Adult Females	5,285	1.71	70	0.129	0.467	3.62

a- Body weights were from US EPA (2014a)
b- TEF: Total Equivalent Residue calculated as the Ratio CPF-oxon PoD to CPF PoD.
c- MOE calculations: CPF-oxon PoD ÷ $DW_{PDP\ or\ EMON}$ Residue
Highlighted are populations of concern for spray drift and aggregate exposure and risk characterization.

V.E.2.b. Risk Characterization of the Drinking Water Exposure:

Table 55 shows acute MOEs for exposure to CPF-oxon in drinking water for the four sentinel populations based on the drinking water residue data from PDP and DPR surface and ground water residues. The MOEs were highest for PDP (18,856 – 47,636) and lowest for surface water (405 – 1,299). All MOEs for acute water-only exposure were greater than the target of 100.

Monitoring and modeling data were not available to estimate the steady-state (21-day) exposure to CPF-oxon in drinking water. If acute exposure estimates are compared to steady-state PoDs, the resulting MOEs would be lower than those shown in Table 55. However, lack of residue data precludes a steady-state drinking water assessment at this time.

Table 55. Acute Exposure Estimates and MOEs for CPF-oxon in Drinking Water; Surface and Ground Water

Acute Exposure Estimates for CPF-Oxon in Drinking Water Based on 2001-2013 PDP Residue Data						
Population Subgroup	Exposure (mg/kg/d)[a]			MOE[b]		
	95th	99th	99.9th	95th	99th	99.9th
All Infants (< 1 year old)	0.000004	0.000061	0.000108	42425	2782	1571
Children 1-2 years old	0.000002	0.000025	0.000057	79555	6364	2791
Children 6-12 years old	0.000002	0.000015	0.000036	71454	9527	3970
Females 13-49 years old	0.000001	0.000017	0.000036	129152	7597	3588
Acute Exposure Estimates for CPF-Oxon in Drinking Water Based on 2005-2014 Surface Water Residue Data						
Population Subgroup	Exposure (mg/kg/d)[a]			MOE[b]		
	95th	99th	99.9th	95th	99th	99.9th
All Infants (< 1 year old)	0.000008	0.000049	0.000419	19875	3469	406
Children 1-2 years old	0.000004	0.000023	0.000177	39750	6913	898
Children 6-12 years old	0.000002	0.000014	0.00011	71500	10214	1300
Females 13-49 years old	0.000002	0.000015	0.000119	63500	8467	1067
Acute Exposure Estimates for CPF-Oxon in Drinking Water Based on 2004-2013 Ground Water Residue Data						
Population Subgroup	Exposure (mg/kg/d)[a]			MOE[b]		
	95th	99th	99.9th	95th	99th	99.9th
All Infants (< 1 year old)	0.000018	0.000127	0.000222	9444	1339	766
Children 1-2 years old	0.000012	0.000054	0.000115	13250	2944	1478
Children 6-12 years old	0.000008	0.000031	0.000075	17875	4613	1907
Females 13-49 years old	0.000009	0.000036	0.000073	14111	3528	1740

a- CPF exposure values were converted to CPF-oxon by applying a molecular weight correction factor (0.9541).
b- MOE calculations: CPF-oxon PoD ÷ DW_{PDP} Residue
Highlighted indicates subgroup with the DW exposure but MOE was within acceptable range.

V.F. Aggregate Exposure: Combined MOEs (Dietary [food only], Drinking Water [PDP or Surface Water], Spray Drift)

When exposure occurs by more than one route and route-specific NOELs are used, a combined MOE for all routes can be calculated. This section is designed to show the acute aggregate MOEs for children (1-2 years old) for all routes (Appendix 2, Table 16) including: combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition); inhalation (I), in addition to dietary (D: food only; PoD = 0.581 mg/kg/d; Table 51) and drinking water (CPF-oxon PoD = 0.159 mg/kg/d).

$$\text{Aggregate MOE} = \frac{1}{\frac{1}{MOE_{CD}} + \frac{1}{MOE_{I}} + \frac{1}{MOE_{D}} + \frac{1}{MOE_{DW\ (PDP\ or\ EMON)}}}.$$

Aggregate exposure MOEs include the parameters described above for children (1-2 years old) as well as the acute drinking water PoD for CPF-oxon of 0.159 mg/kg/d and body weight of 13 kg described in the Exposure Assessment, Section IV.

V.F.1. Aggregate MOEs after Aircraft Exposure from Spray Drift (Children 1-2 years old)

Table 56 has the CPF to CPF-oxon conversion values used in the aggregate risk characterizations for spray drift bystander exposure. Table 56 indicates that once the values for inhalation are added, the aggregate MOEs fall below the target of 100. Additional factors that decrease the aggregate MOEs are increased application volume and increased application rate. As these are increased, the distances where aggregate MOEs are below the target of 100 extend to 1000 feet. Inhalation appears to drive the MOEs below the target value for children (1-2 years old).

Table 56. Dermal and Oral MOEs for Children (1-2 years old) at Various Distances Downwind from Fields Treated with CPF by Aircraft or Helicopter

Application Scenario	Appl. Vol. (gal/acre)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distances Downwind from the Treated Fields						
				10 feet	25 feet	50 feet	100 feet	250 feet	500 feet	1000 feet
Aircraft or Helicopter (Children 1-2 years old)										
AT802A Fixed Wing Aircraft	2	CD[a]	1	127	149	190	282	541	907	1701
			2	63	75	95	143	285	523	1331
			2.3	55	65	83	124	249	469	1210
		CD + I[b]	1	47	53	61	78	116	166	300
			2	26	29	35	46	74	120	264
			2.3	23	27	32	42	69	113	252
		CD + I + D[c]	1	45	51	58	74	107	148	246
			2	25	29	34	44	70	110	221
			2.3	23	26	31	41	65	105	213
		CD + I + D + DW-PDP[e]	1	45	51	58	74	106	147	244
			2	25	29	34	44	70	110	220
			2.3	23	26	31	41	65	104	211
		CD + I + D + DW-EMON[d]	1	43	48	55	68	95	127	193
			2	25	28	32	42	65	98	178
			2.3	22	25	30	39	61	94	172
Bell 205 Helicopter	2	CD	1	100	158	258	424	664	1118	2289
			2	50	78	126	203	367	716	1633
			2.3	43	68	110	176	325	645	1500
		CD + I	1	37	49	65	86	126	192	347

			2	20	27	37	51	85	145	287
			2.3	18	25	34	48	80	140	280
		CD + I + D	1	36	47	62	81	115	169	277
			2	19	26	36	49	80	131	238
			2.3	18	24	33	46	76	127	233
		CD + I + D + DW-PDP	1	36	47	62	81	115	168	274
			2	19	26	36	49	80	131	236
			2.3	18	24	33	46	76	126	231
		CD + I + D + DW-EMON	1	34	45	58	74	102	142	212
			2	19	26	34	47	73	115	188
			2.3	17	24	32	44	70	111	185
AT802A Fixed Wing Aircraft	15	CD	1	147	174	217	325	633	1021	1368
			2	70	83	103	152	288	452	622
			2.3	61	72	89	131	248	390	538
		CD + I	1	39	43	47	56	73	89	115
			2	22	24	27	32	43	55	75
			2.3	19	21	24	29	39	50	69
		CD + I + D	1	38	42	46	54	69	84	106
			2	21	24	26	32	42	53	71
			2.3	19	21	23	28	38	48	66
		CD + I + D + DW-PDP	1	38	42	46	54	69	83	105
			2	21	24	26	31	42	52	71
			2.3	19	21	23	28	38	48	66
		CD + I + D + DW-EMON	1	37	40	44	51	64	77	95
			2	21	23	25	30	40	50	66
			2.3	19	21	23	28	36	46	61
Bell 205 Helicopter	15	CD	1	107	175	301	519	747	996	1521
			2	52	84	141	238	340	478	790
			2.3	45	72	121	204	294	419	692
		CD + I	1	26	33	40	48	59	76	109
			2	17	21	27	33	42	56	84
			2.3	15	19	24	30	39	52	78
		CD + I + D	1	26	32	39	46	57	72	101
			2	16	21	26	33	41	54	79
			2.3	15	19	24	29	38	50	74
		CD + I + D + DW-PDP	1	26	32	39	46	57	72	100
			2	16	21	26	32	41	54	79
			2.3	15	19	24	29	38	50	74
		CD + I + D + DW-EMON	1	25	31	37	44	54	67	91
			2	16	21	26	31	39	51	73
			2.3	14	18	23	29	36	47	68

Source: US EPA (2014a): Dermal PoD-Steady-state = 134.25 mg/kg/d; For calculations, Dermal Absorption (0-1) = 1; Oral PoD Steady-state: 0.099 mg/kg/d. Target MOE = 100

a- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion)

b- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion; inhalation (I))

c- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d).

d- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON).

V.F.2. Aggregate MOEs after Ground Boom Exposure from Spray Drift (Children 1-2 years old)

Aggregate MOEs for this exposure scenario are below the target of 100 for children (1-2 years old) from 75 feet for dermal plus inhalation at 1 lb/ac to 250 ft for all aggregate exposures at 2 lb/ac, 4 lb/ac, and 6 lb/ac (Table 57).

Table 57. Aggregate MOEs after Ground Boom Exposure from Spray Drift (Children 1-2 years old)

Application Scenario	Appl. Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	25 feet	50 feet	75 feet	100 feet	150 feet	200 feet	250 feet
Ground boom (Children 1-2 years old)										
High Boom	40 (50th percentile)	CD[a]	1	2578	3888	5211	6620	9072	11664	14408
			2	1289	1944	2606	3310	4536	5832	7204
			4	645	972	1303	1655	2268	2916	3602
			6	430	648	869	1103	1512	1944	2401
		CD + I[b]	1	79	88	97	106	121	134	146
			2	46	53	60	66	78	88	99
			4	29	33	39	44	54	63	74
			6	22	26	30	35	45	56	66
		CD + I + D[c]	1	74	82	91	98	111	122	132
			2	28	32	38	43	52	60	70
			4	28	32	38	43	52	60	70
			6	21	25	30	34	44	53	63
		CD + D + DW-PDP[d]	1	74	82	91	98	111	121	131
			2	45	51	57	63	73	83	92
			4	28	32	38	42	52	60	70
			6	21	25	30	34	44	53	63
		CD + D + DW-EMON[d]	1	69	76	83	89	99	107	115
			2	43	48	54	59	68	76	83
			4	27	31	36	41	49	57	65
			6	21	25	29	33	42	50	59
Low Boom	40 (50th percentile)	CD	1	4899	7204	9421	12247	16329	20411	24494
			2	2449	3602	4710	6123	8165	10206	12247
			4	1225	1801	2355	3062	4082	5103	6123
			6	816	1201	1570	2041	2722	3402	4082
		CD + I	1	80	89	98	107	122	134	146
			2	47	53	61	67	78	89	99
			4	29	34	39	44	54	64	74
			6	22	26	31	36	46	56	67
		CD + I + D	1	75	83	92	99	112	122	132
			2	46	51	58	64	74	83	93
			4	29	33	38	43	52	61	71
			6	22	26	30	35	44	54	64
		CD + D + DW-PDP	1	75	83	91	99	111	122	132
			2	46	51	58	64	74	83	92
			4	28	33	38	43	52	61	70
			6	22	26	30	35	44	54	64
		CD + D + DW-EMON	1	70	76	83	89	99	108	115
			2	43	49	55	60	68	76	84
			4	28	32	37	41	49	57	65
			6	21	25	29	34	42	51	60
High Boom	40 (90th percentile)	CD	1	1814	2525	3266	4082	5443	6804	8165
			2	907	1263	1633	2041	2722	3402	4082
			4	454	631	816	1021	1361	1701	2041
			6	302	421	544	680	907	1134	1361
		CD + I	1	78	87	96	105	120	133	145
			2	46	52	59	66	77	87	98
			4	28	33	38	43	53	62	73
			6	21	25	30	35	44	54	65
		CD + I + D	1	74	82	90	98	110	121	131
			2	44	50	57	63	73	82	91
			4	27	32	37	42	51	60	69
			6	21	25	29	34	43	52	62
		CD + D + DW-	1	73	81	90	97	110	121	130

		PDP	2	44	50	57	62	73	82	91
			4	27	32	37	42	51	59	69
			6	21	25	29	34	43	52	62
		CD + D + DW-EMON	1	68	75	82	88	98	107	114
			2	42	47	53	58	67	75	83
			4	27	31	36	40	48	56	64
			6	20	24	28	33	41	49	58
Low Boom	40 (90th percentile)	CD	1	2882	3951	5103	6280	8446	10206	12247
			2	1441	1975	2551	3140	4223	5103	6123
			4	720	988	1276	1570	2112	2551	3062
			6	480	658	850	1047	1408	1701	2041
		CD + I	1	79	88	97	106	121	134	145
			2	47	53	60	66	78	88	98
			4	29	33	39	44	54	63	73
			6	22	26	30	35	45	55	66
		CD + I + D	1	75	83	91	98	111	122	132
			2	45	51	57	63	73	83	92
			4	28	32	38	42	52	60	70
			6	21	25	30	34	44	53	63
		CD + D + DW-PDP	1	74	82	91	98	111	121	131
			2	45	51	57	63	73	83	92
			4	28	32	38	42	51	60	70
			6	21	25	30	34	44	53	63
		CD + D + DW-EMON	1	69	76	83	89	99	107	115
			2	43	48	54	59	68	76	83
			4	27	31	36	41	49	56	65
			6	21	25	29	33	42	50	59

Source: US EPA (2014a): Dermal PoD-Steady-state = 134.25 mg/kg/d; For calculations, Dermal Absorption (0-1) = 1; Oral PoD Steady-state: 0.099 mg/kg/d. Target MOE = 100

a- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion)

b- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion; inhalation (I))

c- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d).

d- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON).

V.F.3. Aggregate MOEs after Orchard Airblast Exposure from Spray Drift (Children 1-2 years old)

Both orchard airblast scenarios show that dermal MOES are below 100 only at the highest application rates (lb/acre). When inhalation is added the aggregate MOEs are below 100 at 75 ft for 1 lb/ac and at 250 ft for all other application rates (Table 58).

Table 58. Dermal and Oral MOEs for Children (1-2 years old) at Various Distances Downwind from Fields Treated with CPF by Orchard Airblast

Application Scenario	Appl. Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	25 feet	50 feet	75 feet	100 feet	150 feet	200 feet	250 feet
Orchard Airblast (Children 1-2 years old)										
Dormant Apples	60	CD[b]	1	443	1163	2371	4173	9876	18697	31005
			2	221	582	1186	2086	4938	9349	15502
			4	111	291	593	1043	2469	4674	7751
			6	74	194	395	695	1646	3116	5167
		CD + I[c]	1	69	83	95	105	121	134	147
			2	39	50	58	66	78	89	99
			4	23	31	37	43	54	64	75
			6	17	24	29	35	45	56	67
		CD + I + D[d]	1	65	79	89	98	111	122	132
			2	38	48	56	63	74	83	93
			4	23	30	36	42	52	61	71
			6	17	23	29	34	44	54	64
		CD + D + DW-PDP[e]	1	78	89	97	111	122	132	141
			2	38	48	56	62	73	83	92
			4	23	30	36	42	52	61	71
			6	17	23	29	34	44	54	64
		CD + D + DW-EMON[e]	1	61	72	81	88	99	108	115
			2	37	45	53	59	68	76	84
			4	23	29	35	40	49	57	66
			6	17	23	28	33	42	51	60
Sparse Orchard	60	CD	1	546	1198	2134	3342	6567	10886	16221
			2	273	599	1067	1671	3283	5443	8111
			4	136	300	533	835	1642	2722	4055
			6	91	200	356	557	1094	1814	2704
		CD + I	1	71	84	95	104	120	134	146
			2	41	50	58	65	77	88	99
			4	24	31	37	43	53	63	74
			6	18	24	29	34	45	55	66
		CD + I + D	1	67	79	89	97	111	122	132
			2	40	48	56	62	73	83	92
			4	24	30	36	41	51	60	70
			6	18	23	28	33	43	53	63
		CD + D + DW-PDP	1	67	79	88	97	110	121	131
			2	40	48	56	62	73	83	92

			4	24	30	36	41	51	60	70
			6	18	23	28	33	43	53	63
		CD + D + DW-EMON	1	63	72	81	88	98	107	115
			2	38	46	52	58	68	76	84
			4	23	29	35	40	48	57	65
			6	18	23	27	32	41	50	59

Source: US EPA (2014a): Dermal PoD-Steady-state = 134.25 mg/kg/d; For calculations, Dermal Absorption (0-1) = 1; Oral PoD Steady-state: 0.099 mg/kg/d. Target MOE = 100

a- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion)

b- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion; inhalation (I))

c- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d).

d- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON).

VI. RISK APPRAISAL

VI.A. Introduction

The risk assessment reported here 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.

Studies on potential adverse effects after acute, subchronic or chronic oral, dermal or inhalation exposure in animals have focused on ChE inhibition in plasma, RBCs, and the brain. Controlled dosing studies that measured RBC and plasma ChE in humans are available (Eaton et al., 2008). RBC AChE inhibition is commonly used as a surrogate of cholinesterase inhibition in target tissues in the central and peripheral nervous system (Furman, 2010; US EPA, 2014a). A 10% inhibition is the lowest level of cholinesterase inhibition which can be reliably measured. For this risk assessment, the PBPK-PD model which incorporates human data was used to estimate PoDs based on 10% RBC AChE inhibition. Other potentially noncholinergic effects and uncertainties in using the PBPK-PD model are discussed below.

VI.B. Uncertainties Associated with the Hazard Identification

VI.B.1. The PBPK-PD Model

HHA adopted the critical PoDs for CPF from the 2014 US EPA revised human health assessment. The PBPK-PD model was used to estimate these values for10% RBC AChE inhibition in various human populations, durations and routes. This model has been in development for the last 15 years and has undergone numerous scientific evaluations (US EPA/SAP, 2008; US EPA/SAP, 2010; US EPA/SAP, 2012; US EPA, 2014a)as well as publications (Timchalk et al., 2002a; Timchalk et al., 2002b; Timchalk et al., 2005; Timchalk et al., 2006; Timchalk and Poet, 2008; Smith et al., 2011; Poet et al., 2014; Smith et al., 2014; Poet et al., 2017a). The discussion below focuses mainly on the uncertainties with the model used by US EPA in 2014 (US EPA, 2014a), however, predictions by the updated 2017 model (Poet et al., 2017a) are included for comparison when appropriate.

The PBPK-PD model is based on the pharmacokinetics of CPF in two human dosing studies and a human dermal dosing study. Human liver microsomes and plasma were used to represent CPF metabolic variability across a broad range of ages (Nolan et al., 1984; Smith et al., 2011; Poet et al., 2014; Smith et al., 2014; US EPA, 2014a; Poet et al., 2017a).

The model predicts a time-course of CPF metabolism and RBC AChE inhibition, reactivation, and regeneration after oral, dermal, and inhalation exposure to CPF. It has been reviewed and validated with human data since publication of the original PBPK model (Timchalk et al., 2002b). One of the main advantages of this model is the availability of human volunteer dosing studies (Nolan et al., 1984; Vaccaro et al., 1993; Kisicki et al., 1999) and sources of well-characterized human tissues (Smith et al., 2011). The model incorporates life-stages for infants (6 months), children (3-year-olds), and adults (30 year olds) as well as pregnancy parameters (Smith et al., 2011; Smith et al., 2014; Poet et al., 2017) and multi-route human exposure parameters (oral, dermal and inhalation) (Poet et al., 2014). The 2017 updated model includes sensitivity analyses for each of the 120-160 parameters to determine those that drive the greatest variability within the model (e.g. chlorpyrifos activation and deactivation reactions) as well as uncertainty calculations (Poet et al., 2017a).

VI.B.1.a. Acute Oral PoDs from the PBPK-PD Model

The PBPK-derived acute oral PoDs ranged from 0.5-0.6 mg/kg/day for the evaluated population subgroups including infants, children and women of childbearing age. HHA used these values to characterize the human risk to CPF from acute exposure from food and drinking water. These PoDs were similar to the acute NOELs established in the available animal studies (0.4-0.5 mg/kg/day) for RBC AChE inhibition. The overall database for chlorpyrifos generally shows that the threshold dose for RBC AChE inhibition is around 1 mg/kg/day, including that for young rats.

VI.B.1.b. Steady-State Oral PoDs from the PBPK-PD Model

Separate subchronic and chronic oral PoDs were not specifically calculated in the PBPK-PD model reported in the current US EPA (2014a) IRED. Instead the model generated a 21-day steady-state oral PoD for 10% RBC AChE inhibition in humans. Repeated exposures result in a balance between inhibition and generation of new AChE. Studies of 14-21 day durations show AChE inhibition to the same degree as those of longer duration (US EPA, 2014a). The model-derived steady state human PoDs were in the range of the NOELs from repeated dosing from several weeks to 2 years (0.03-0.05 mg/kg/day) in animal studies.

VI.B.1.c. Steady-State Dermal, Non-Dietary Ingestion and Inhalation PoDs from the PBPK-PD Model

PoDs for steady-state dermal, non-dietary ingestion and inhalation exposures were adopted from the PBPK-PD model presented by US EPA (2014b). The US EPA model was based on the level of RBC ChE inhibition in humans achieved at or before 21 days of daily inhalation exposure. These values were used to calculate risks to children and females of childbearing age from spray drift near application sites, as well as risks associated from aggregate exposures.

Spray drift exposure is of short-term duration (1 – 1.5 hours) for which acute PoDs would normally be used to estimate relevant risks. However, this practice may underestimate risks to individuals residing in areas of high CPF use because acute PoDs do not by themselves account for the elevated level of AChE inhibition already present in such populations. Indeed, enzyme activities in children residing in high CPF use areas are decreased by about 30% compared to

children who live in non- or low-use agricultural areas. This is evident in a study by Kapka-Skrzypczak et al. (2015) who compared RBC AChE levels (adjusted for hemoglobin concentration, Hb) in a group of Polish children (8-12 years old) living in a high pesticide use area versus matched children in a lower pesticide use area. The study did not specify the pesticides involved however at least one AChE inhibiting pesticide was detected in sweat sorbents from the children.

AChE (mU/µmol Hb)			
n (sex)	**mean**	**SD**	**CV (%)**
Exposed			
49 (M)	243.40	28.17	11.6
59 (F)	240.02	25.52	10.6
Controls			
47 (M)	349.59	50.19	14.4
45 (F)	346.91	44.29	12.8

In addition, Suarez-Lopez et al. (2013) made a similar observation in children who lived with a household member who worked at a flower plantation but lived at varying distances from the plantations. This study also did not specify the pesticides involved.

AChE (U/ml)			
1st Tertile:			
67% cohabited with flower worker, 360m avg distance to flower plantation			
n (sex)	**mean**	**SD**	**CV (%)**
104 (M/F)	2.63	0.27	10.3
3rd Tertile:			
45% cohabited with flower worker, 501m avg distance to flower plantation			
n (sex)	**mean**	**SD**	**CV (%)**
102 (M/F)	3.67	0.29	7.9

Therefore, when evaluating the risk from short term exposures in the presence of concurrent background levels of inhibition likely to occur in populations from areas of high CPF use, we considered three factors to be critical: (1) AChE inhibition sustained by constant exposure is cumulative; (2) Complete recovery of enzyme activity in humans is not achieved even after 10 days of non-exposure; and (3) AChE inhibition in laboratory animals subjected to repeated doses of CPF reaches steady state levels after ~2-3 weeks of exposure. In this light, we concluded that the effect produced from short term drift exposures would be most prudently characterized by a PoD derived from repeated (21-day) dosing.

VI.C. Uncertainties Related to Exposure Assessment

VI.C.1. Acute CPF Spray Drift Exposure Uncertainty

This exposure assessment employed state-of-the-art computer models (AgDRIFT and AGDISP) coupled with the latest version of the US EPA Residential Exposure Assessment Standard Operating Procedures for characterizing the non-occupational bystanders' exposure to spray drift of CPF. Accordingly, the intrinsic uncertainties associated with these modeling and exposure computational methodologies (e.g., assumptions) will be translated into the bystanders' exposure

estimates of CPF based on the manner in which these computer models and SOPs were applied. The intrinsic uncertainties associated with these computer models and SOPs have been detailed in the original documentations (Teske *et al.*, 2002b; Teske and Curbishley, 2013)US EPA 2012c). Therefore, the focus of the following discussion is to evaluate the uncertainties of exposure estimates based on the approach of which these computer models and exposure computations were performed.

For modeling spray drift, the input parameters were tailored to match the actual field operation and meteorological conditions that are expected to result in the reasonable worst-case horizontal deposition and air concentration estimates under California use conditions (Appendix 2) (Barry, 2017). Hence, these aerial application exposure estimates of CPF can be considered as reasonable worst-case estimates of exposures under California conditions. Unlike the aerial application, the available spray drift computer models are unable to generate air concentrations of CPF associated with ground boom and orchard airblast applications. To account for inhalation exposures in the orchard airblast and ground boom application methods, this exposure assessment used surrogate air concentrations estimates obtained by modeling aerial applications using the AT802A aircraft. These surrogate air concentrations are likely reasonable worst case air concentration estimates for orchard airblast and ground boom. As a point of comparison, the California Air Resources Board (CARB) has conducted two CPF application site air monitoring studies: CARB (2016) and CARB (1998). The CARB (2016) study measured air concentration associated with a helicopter application. The results of the CARB (2016)study are not used for comparison for the following reasons: 1) the sampling method was best suited for collecting vapor so it was not optimal for collecting aerosols that comprised spray drift during an application (further discussed below), 2) the application period sampling interval did not match the actual application time, and 3) the maximum measured air concentration was not collected at the sampler located in the predominant wind direction. CARB (1998)measured air concentrations of CPF during and after an orchard airblast application to an orange orchard in Tulare, CA. This study measured air concentrations during two separate application periods using an air monitoring method best suited for collecting vapor. Spray drift is composed of aerosols and requires a different sampling method to adequately characterize air concentrations (Streicher et al., 1994). Therefore, the CARB (1998)air monitoring results cannot be definitively compared to the AGDISP air concentration estimates, but general observations can be made. The air concentrations in the study were measured over several days, with two application periods sampled. Those two application sampling periods are well described and correctly bracketed the actual application period. Therefore, they are the appropriate periods to compare to the AGDISP estimated air concentrations. The CARB measured air concentrations must be adjusted to the same averaging time as the modeled air concentrations using the peak-to-mean method as described in Barry (2000). The AGDISP model produces 1 hr time weighted average air concentration estimates. The CARB (1998) application sampling interval peak air concentrations adjusted to 1-hr time weighted average concentrations are 0.06 mg/m^3 and 0.08 mg/m^3 for application periods 1 and 2, respectively. These measured values are similar to the AGDISP female 13-49 year old air concentration of 0.06 mg/m^3 at 25 ft and 0.05 mg/m^3 at 50 ft and 1-2 year old child air concentration of 0.08 mg/m^3 at 25 ft and 0.07 mg/m^3 at 50 ft CARB (1998) measured air concentrations were sampled at 30 ft and 57 ft from the application edge. This general comparison suggests that the surrogate aerial air concentrations are reasonable estimates of inhalation exposures associated with orchard airblast applications. In general, it is likely that

the air concentrations estimated for the fixed-wing aircraft are as high or higher, than those associated with either ground boom or orchard airblast because of the higher ground speed and the higher release height of the spray from aircraft.

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, this value is comparable to the TTR value obtained in California (0.124 ± 0.004). In fact, risk estimates based on TTR data from Mississippi and California are essentially identical (see Tables 59 and 60).

Table 59. MOEs for Children (1-2 years old) Associated with Spray Drift at Various Distances from a Field Treated with CPF Using Aerial Equipment and the Mississippi turf transferable residue (TTR) value from Stafford and Robb (1999)

Application Scenario	Appl. Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distances Downwind from the Treated Fields						
				10 feet	25 feet	50 feet	100 feet	250 feet	500 feet	1000 feet
Aircraft or Helicopter (Children 1-2 years old)										
AT802A Fixed Wing Aircraft	2	CD[b]	1	109	128	163	242	463	777	1458
			2	54	64	82	122	244	448	1141
			2.3	47	56	71	107	213	402	1037
		CD + I[c]	1	44	50	58	74	112	161	292
			2	24	27	33	44	71	115	255
			2.3	22	25	30	40	66	109	243
		CD + I + D[d]	1	43	48	56	71	103	144	241
			2	24	27	32	42	67	106	215
			2.3	22	24	29	39	63	101	207
		CD + I + D + DW-PDP[e]	1	43	48	55	71	103	143	239
			2	24	27	32	42	67	106	214
			2.3	22	24	29	39	63	101	205
		CD + I + D + DW-EMON[e]	1	41	46	52	66	93	124	190
			2	23	26	31	40	63	95	174
			2.3	21	24	28	37	59	91	168
Bell 205 Helicopter	2	CD	1	86	135	221	363	569	958	1962
			2	42	67	108	174	314	614	1399
			2.3	37	58	94	151	278	553	1285
		CD + I	1	35	46	62	83	122	187	338
			2	18	25	35	49	82	141	279
			2.3	17	23	32	46	77	135	272
		CD + I + D	1	34	45	59	79	112	165	271
			2	18	25	34	48	77	128	232
			2.3	17	23	32	44	73	123	227
		CD + I + D + DW-PDP	1	34	45	59	78	111	164	269
			2	18	25	34	47	77	127	230
			2.3	17	23	32	44	73	122	225
		CD + I + D + DW-EMON	1	32	43	56	72	99	139	208
			2	18	24	33	45	71	112	184
			2.3	16	22	30	42	68	108	181
AT802A Fixed Wing Aircraft	15	CD	1	126	149	186	278	542	875	1173
			2	60	71	88	130	247	387	533
			2.3	52	62	76	112	213	334	461
		CD + I	1	38	41	46	55	71	88	113
			2	21	23	26	31	42	54	73

			2.3	18	20	23	28	38	49	68
		CD + I + D	1	37	40	44	53	68	83	104
			2	20	23	25	30	41	52	70
			2.3	18	20	22	27	37	47	65
		CD + I + D + DW-PDP	1	36	40	44	53	68	82	104
			2	20	23	25	30	41	51	69
			2.3	18	20	22	27	37	47	64
		CD + I + D + DW-EMON	1	35	38	42	50	63	76	94
			2	20	22	24	29	39	49	65
			2.3	18	20	22	27	35	45	60
Bell 205 Helicopter	15	CD	1	92	150	258	445	640	853	1304
			2	45	72	121	204	292	410	677
			2.3	39	62	104	175	252	359	593
		CD + I	1	25	32	39	47	59	75	107
			2	16	20	26	33	42	55	83
			2.3	14	18	23	29	38	51	77
		CD + I + D	1	25	31	38	46	56	71	100
			2	16	20	26	32	40	53	78
			2.3	14	18	23	29	37	49	73
		CD + I + D + DW-PDP	1	25	31	38	46	56	71	99
			2	16	20	26	32	40	53	78
			2.3	14	18	23	29	37	49	73
		CD + I + D + DW-EMON	1	24	30	36	43	53	66	90
			2	15	20	25	31	39	50	72
			2.3	14	18	22	28	35	46	67

a- From US EPA (2014a): Dermal PoD-Steady-state = 134.25 mg/kg/d; For calculations, Dermal Absorption (0-1) = 1; Oral PoD Steady-state: 0.099 mg/kg/d. Target MOE = 100

b- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion)

c- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion; inhalation (I))

d- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d).

e- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON).

Table 60. Aggregate MOEs for Children (1-2 years old) at Various Distances Downwind from Fields Treated with CPF by Aircraft or Helicopter Using California Turf Transferable Residue (TTR) from Stafford and Robb (1999)

Application Scenario	Appl. Vol. (gallon/acre)	Exposure Route	Appl. Rate (lb/acre)	MOE at Various Distances Downwind from the Treated Fields						
				10 feet	25 feet	50 feet	100 feet	250 feet	500 feet	1000 feet
Aircraft or Helicopter (Children 1-2 years old)										
AT802A Fixed Wing Aircraft	2	CD[b]	1	127	149	190	282	541	907	1701
			2	63	75	95	143	285	523	1331
			2.3	55	65	83	124	249	469	1210
		CD + I[c]	1	47	53	61	78	116	166	300
			2	26	29	35	46	74	120	264
			2.3	23	27	32	42	69	113	252
		CD + I + D[d]	1	45	51	58	74	107	148	246
			2	25	29	34	44	70	110	221
			2.3	23	26	31	41	65	105	213
		CD + I + D + DW-PDP[e]	1	45	51	58	74	106	147	244
			2	25	29	34	44	70	110	220
			2.3	23	26	31	41	65	104	211
		CD + I + D + DW-EMON[e]	1	43	48	55	68	95	127	193
			2	25	28	32	42	65	98	178
			2.3	22	25	30	39	61	94	172
Bell 205 Helicopter	2	CD	1	100	158	258	424	664	1118	2289
			2	50	78	126	203	367	716	1633
			2.3	43	68	110	176	325	645	1500
		CD + I	1	37	49	65	86	126	192	347
			2	20	27	37	51	85	145	287
			2.3	18	25	34	48	80	140	280
		CD + I + D	1	36	47	62	81	115	169	277
			2	19	26	36	49	80	131	238
			2.3	18	24	33	46	76	127	233
		CD + I + D + DW-PDP	1	36	47	62	81	115	168	274
			2	19	26	36	49	80	131	236
			2.3	18	24	33	46	76	126	231
		CD + I + D + DW-EMON	1	34	45	58	74	102	142	212
			2	19	26	34	47	73	115	188
			2.3	17	24	32	44	70	111	185
AT802A Fixed Wing Aircraft	15	CD	1	147	174	217	325	633	1021	1368
			2	70	83	103	152	288	452	622
			2.3	61	72	89	131	248	390	538
		CD + I	1	39	43	47	56	73	89	115
			2	22	24	27	32	43	55	75
			2.3	19	21	24	29	39	50	69
		CD + I + D	1	38	42	46	54	69	84	106
			2	21	24	26	32	42	53	71
			2.3	19	21	23	28	38	48	66
		CD + I + D + DW-PDP	1	38	42	46	54	69	83	105
			2	21	24	26	31	42	52	71
			2.3	19	21	23	28	38	48	66
		CD + I + D + DW-EMON	1	37	40	44	51	64	77	95
			2	21	23	25	30	40	50	66
			2.3	19	21	23	28	36	46	61
Bell 205 Helicopter	15	CD	1	107	175	301	519	747	996	1521
			2	52	84	141	238	340	478	790
			2.3	45	72	121	204	294	419	692
		CD + I	1	26	33	40	48	59	76	109
			2	17	21	27	33	42	56	84

			2.3	15	19	24	30	39	52	78
		CD + I + D	1	26	32	39	46	57	72	101
			2	16	21	26	33	41	54	79
			2.3	15	19	24	29	38	50	74
		CD + I + D + DW-PDP	1	26	32	39	46	57	72	100
			2	16	21	26	32	41	54	79
			2.3	15	19	24	29	38	50	74
		CD + I + D + DW-EMON	1	25	31	37	44	54	67	91
			2	16	21	26	31	39	51	73
			2.3	14	18	23	29	36	47	68

a- From US EPA (2014a): Dermal PoD-Steady-state = 134.25 mg/kg/d; For calculations, Dermal Absorption (0-1) = 1; Oral PoD Steady-state: 0.099 mg/kg/d. Target MOE = 100
b- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion)
c- Combined Deposition (CD = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion; inhalation (I))
d- Combined Deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d).
e- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; PoD = 0.581 mg/kg/d); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON). Target MOE = 100

VI.C.2. Dietary Exposure Uncertainties

Issues Related to Food Exposure:

Illegal Residues In Food Were Not Included In The Exposure Assessment: 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 2012 to 2014, DPR's California Pesticide Residue Monitoring Program (CPRMP) analyzed 2180 food samples and detected 63 (3% of total samples) illegal chlorpyrifos residues on the commodities shown in Table 61. A high proportion of illegal detections were on cactus (leaves or fruit), litchi, and longan. Most or all of these foods were imported. Certain population ethnic subgroups (e.g., Hispanic and Asian) in California have higher consumption of these foods. From 2015 to 2017, CPRMP analyzed over 2500 samples of fresh produce, of which 269 (11%) contained illegal CPF residues. Litchi, orange, oriental pear, cactus and tangelo were among the produce with frequent illegal detections. HHA evaluations of these cases concluded that 23 (about 1% of 2500 samples) were of potential health risk to consumers.

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 61 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 California Pesticide Residue Monitoring Program, 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 State of California's 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 61. Commodities Sampled by DPR's Pesticide Residue Monitoring Program that had Illegal Chlorpyrifos Residues from January 2015 to November 2017

Commodity Name	Samples Tested	Samples with Illegal Residues[b]	% with Illegal Residues	Samples with Illegal Residues[a]		
				Minimum Conc. (ppm)	Maximum Conc. (ppm)	Average Conc. (ppm)
ARROWHEAD (SAGITTARIA SPP.)	1	1 (1)	100	0.032	0.032	0.032
ASPARAGUS (SPEARS, FERNS, ETC.)	73	3	4	0.023	0.140	0.078
BANANA	151	22	15	0.010	0.090	0.031
BEANS (GREEN, STRING)	56	2	4	0.022	0.068	0.045
BOK CHOY (WONG BOK)	24	1	4	0.028	0.028	0.028
CHAYOTE (CHRISTOPHENES)	69	2	3	0.014	0.022	0.018
CHINESE RADISH/DAIKON (LOBOK, JAPANESE RADISH)	27	1	4	0.034	0.034	0.034
KALE	223	2	1	0.022	0.023	0.023
KIWI FRUIT	67	2	3	0.017	0.023	0.020
LEMON	78	7	9	0.013	0.100	0.046
LIME (MEXICAN LIME, ETC.)	81	4	5	0.026	0.039	0.033
LITCHI NUTS	25	15 (9)	60	0.029	0.370	0.117
LONGAN (LONGAN FRUIT)	30	7 (2)	23	0.022	0.110	0.059
NECTARINE	213	3	1	0.022	0.038	0.030
ORANGE (ALL OR UNSPEC)	219	56	26	0.013	0.120	0.048
ORANGE, SWEET	27	4	15	0.026	0.068	0.038
PASSION FRUIT (TAMARILLO, PURPLE GRANADILLA)	2	1 (1)	50	0.020	0.020	0.020
PEAR	55	1	2	0.047	0.047	0.047
PEAR, ASIAN (ORIENTAL PEAR)	63	18 (4)	29	0.022	0.220	0.069
PEPPERS (ALL OR UNSPEC)	2	1	50	0.025	0.025	0.025
PEPPERS (CHILI TYPE) (FLAVORING AND SPICE CROP)	214	26	12	0.011	0.270	0.059
PEPPERS (FRUITING VEGETABLE), (BELL,CHILI, ETC.)	285	20	7	0.011	0.290	0.099
PERSIMMON, COMMON	6	1	17	0.140	0.140	0.140
PINEAPPLE (FRESH MKT.	33	1	3	0.021	0.021	0.021

Commodity Name	Samples Tested	Samples with Illegal Residues[b]	% with Illegal Residues	Samples with Illegal Residues[a]		
				Minimum Conc. (ppm)	Maximum Conc. (ppm)	Average Conc. (ppm)
PINEAPPLE)						
PRICKLYPEAR (CACTUS PEAR)	31	10 (1)	32	0.012	0.130	0.044
PRICKLYPEAR CACTUS PADS	90	9 (5)	10	0.045	0.160	0.091
RADISH	27	1	4	0.023	0.023	0.023
RADISH TOPS	28	4	14	0.038	0.320	0.155
SUBTROPICAL AND TROPICAL FRUIT (ALL OR UNSPEC)	12	2	17	0.022	0.076	0.049
TANGELO	13	3	23	0.027	0.060	0.047
TANGERINE (MANDARIN, SATSUMA, MURCOTT, ETC.)	194	30	15	0.021	0.180	0.066
TARO (DASHEEN) (ROOT CROP) (WETLAND, UPLAND, ETC.)	8	1	13	0.024	0.024	0.024
TOMATILLO	111	5	5	0.020	0.073	0.042
TURNIP (TURNIP ROOTS)	4	1	25	0.027	0.027	0.027
TURNIPS (ALL OR UNSPEC)	5	2	40	0.028	0.160	0.094
Grand Total	**2547**	**269 (23)**	**11**			

[a] An illegal residue is one that either exceeds the US tolerance or is detected on a commodity that has no tolerance for the subject pesticide

[b] Deemed "potential health risk"

Dietary Risks Evaluated on a Per Capita Basis Rather than Per User: In this risk document, RAS calculated the risk from chlorpyrifos exposure from food using the 2014 US EPA exposure values which were estimated on a per capita basis (all individuals surveyed). RAS selects per user-day basis (consumers only or the population that is exposed) for the acute exposure rather than the entire population (per capita) (CDPR, 2009). In many exposure scenarios, per capita risks would be lower than per user risks. However, since chlorpyrifos is used on such a wide variety of crops, almost everyone in the population can potentially be exposed, so per capita dietary risk is expected to be close to per user dietary risk.

Per capita consumption rates may underestimate the CPF exposure from certain foods such as infant formula to non-nursing infants. The sensitivity analysis of food consumption by the various infant population subgroups in DEEM-FCID v3.16 revealed that the exposure estimates at the 95th percentile were slightly higher for non-nursing infants compared to all infants. However at the 99.9th percentile, the exposure estimates for non-nursing infants and all infant users were essentially the same.

Issues Related to Drinking Water Exposure: US EPA modeling of surface water residues predicted that certain chlorpyrifos uses may result in residue levels exceeding the DWLOC at labeled application rates, including scenarios for California grown crops. Surface water modeling results also suggested that the highest exposures may be localized in small watersheds where

high percent crop treated area could occur. However, EDWC of chlorpyrifos was not modeled under California-specific conditions.

HHA estimated drinking water probabilistic exposures using 1) PDP residue data for chlorpyrifos oxon in treated drinking water in California or 2) monitoring data for chlorpyrifos in surface and ground water in California, and drinking water consumption records in DEEM-FCID. The analyses showed that exposures estimated from residues in surface water could be up to 4-fold higher than exposures estimated from residues in treated drinking water.

PDP is not designed to detect peak concentrations of chlorpyrifos or chlorpyrifos-oxon in drinking water and the estimated exposures were based entirely on LODs. Overall, use of PDP data may lead to an underestimation of actual drinking water exposure.

The DPR surface and ground water programs monitor pesticide residues in water, identify the sources of the contamination, and develop mitigation options for protection of aquatic and human health. These programs are designed to capture higher concentrations coinciding with runoff timing, storm events, high-use regions, and application timing. The DPR monitoring programs detected high residue levels in samples collected from various water sources including irrigation ponds, sloughs, and agricultural drains that are not normally used as sources for drinking water. Consequently, a drinking water exposure based on these residues would likely represent a conservative high end potential exposure. Regardless of the residue database, all acute drinking water MOEs at the 99.9^{th} percentile exposure were substantially higher than the target of 100, ranging between 405 and 3,970. As such, a health concern is not indicated. In conclusion, the actual exposure to chlorpyrifos in the California drinking water is likely to be somewhere between the high end exposure scenario based on the DPR surface and ground water detections and the scenario based on LOD for chlorpyrifos oxon from the PDP monitoring.

Assessing exposures via the lactational pathway: Presently, there are very few studies that have measured CPF concentrations in breast milk of mothers in the US. Each of these studies has its limitations. The results from Weldon et al. (2011) were considered to be the most reliable estimate of breast milk residues for US women. These data can be used to evaluate exposure to CPF from human breast milk to nursing infants when consumption data from NHANES or other sources become available. HHA will continue to follow the literature on pesticide residues in human milk and consumption to address pesticide exposure via the lactational pathway.

Assessing risk from aggregate exposure: In this draft assessment, the aggregate MOE associated with dietary and drinking water exposures was calculate using acute PoD values. As detailed in section VI.B.1.c. of this document, it is evident that people living in high pesticide use area have lower levels of RBC AChE activity than those living in low or no use areas. Therefore, the use of acute PoD may underestimate the aggregate risk.

VI.D. Uncertainties in the Risk Characterization

VI.D.1 Interspecies UF:

The input parameters in the PBPK-PD model were specific for human metabolic and physiological processes. HHA reviewed the evaluations of the model by US EPA and other scientific groups and agrees with the conclusion that the derived human parameters adequately

predict AChE inhibition in controlled human dosing studies and support the reduction of the default interspecies UF of 10 to 1.Comparison of the human and animal NOELs from the available literature also suggest that humans are not more sensitive than animals with respect to ChE inhibition. Nevertheless, we recognize that model systems are not designed to account for all physiological processes that influence xenobiotic concentrations at the target site.

VI.D.2 Intraspecies UF:
The 2014 US EPA PBPK-PD model is not designed to account for all physiological changes during pregnancy. The model published in 2017 was updated to characterize maternal changes during pregnancy, including increased respiration, cardiac output and blood volume (both plasma and RBC), increased glomerular filtration, potential changes in metabolism, enlarged uterus, breasts, and fetal growth (Poet et al., 2017a). However, concerns exist for the updated model as raised by SciPinion reviewers about the model capabilities to estimate AChE inhibition in the fetus and neonate (Oliver et al., 2017).

The main parameters responsible for inter-individual variation in RBC acetylcholinesterase inhibition are related to metabolic clearance of CPF and CPF-oxon. In the PBPK-PD model, predictions of all human age-dependent variability based on hepatic P450 metabolism of CPF to the oxon and subsequent plasma and hepatic PON1 detoxification of CPF-oxon to TCPy were derived from a small sample size. These included 30 human liver microsomes and plasma samples from 20 individuals ranging in age from 13 days old to 75 years old. Adult samples were selected to match adult population distributions for the primary CPF metabolizing P450s (CYP1A2, 3A4/5, 2B6, 2C19). Nevertheless, the small sample size was compensated in the model by using bootstrapping from the raw data and Monte Carlo simulations that increased the variability by up to 10-fold for the critical parameters (see Table 5 earlier in this document).

The liver enzyme activities incorporated into the PBPK-PD model were described in Smith et al. (2011). The liver microsomes were obtained from human cryopreserved tissues. There is concern that these tissues are not representative of live tissues due to the potential for enzyme degradation before or after death. However, the human livers were collected and flash cryopreserved following procedures for organ transplant. Human microsomal fractions were then prepared from these cryopreserved livers following standardized protocols (https://www.xenotech.com/products/subcellular-fractions/human/liver/ microsomes; Rewerts, C., Maciej Czerwinski, M. and Loewen, G. personal communication). Several studies have indicated that PON1 activity is relatively stable during an extended tissue collection time, with liver enzyme functionality declining by less than 30% after 12 hours at room temperature (Gonzalvo et al., 1998) and remaining stable for many years in frozen samples (Huen *et al.*, 2009); https://www.xenotech.com/products/subcellular-fractions/human/liver/microsomes). Utilization of microsomes derived from human tissues is described and recommended in the Federal Food and Drug Administration specifically for use in PBPK modeling (FDA, 2012). However, there are no measured T_0 activity levels for fresh versus preserved human liver microsomes, so the comparative metabolic processes may not be perfectly concordant.

Based on Poet et al. (2017a), the acute oral PoDs used in this risk assessment appear to be the median values for 10% RBC AChE inhibition in non-pregnant females (ED_{10}). The updated 2017 PBPK-PD model also provided ED_{10} values for two other simulated populations (Poet et al.,

2017a): pregnant females and infants. Compared to the respective median values (i.e., 50th percentile), the calculated ED_{10} values based on 10% RBC AChE inhibition at the 1st percentile are about 3-fold lower for pregnant females and 4-fold lower for infants. Therefore, if ED_{10} values at the 1st percentile were used, the associated risks would be up to 4-fold higher.

VI.D.2.a. The Role of Plasma ChE (BuChE) and Neurodevelopment

CPF has been shown to affect plasma/BuChE during development in numerous studies described earlier. Plasma ChE is involved in embryonic development of both neural and extraneural tissues (Brimijoin and Koenigsberger, 1999; Mack and Robitzki, 2000). Importantly, plasma ChE has been shown to be inhibited in animal studies at doses equal to or less than RBC AChE (Marty and Andrus, 2010). Zheng et al. (2000)demonstrated greater BuChE inhibition than RBC AChE in rat neonates after both acute and repeated dose administration of CPF.

A study with gene-targeted mice deficient in AChE ($AChE^{-/-}$) showed that BuChE and likely other enzymes may have assumed the function of AChE during early development (Li et al., 2000a; Xie et al., 2000). The $AChE^{-/-}$ mice showed no physical defects at birth. Their organs and blood cells showed no morphological abnormalities. Electron microscopic examination of the neuromuscular junctions showed normal morphology. Interestingly, BuChE levels in the tissues were similar to those in the wild-type and AChE heterozygous mice. In addition, in the absence of AChE, plasma /BuChE was apparently essential for vital functions. When $AChE^{-/-}$ mice were treated with bambuterol, a specific plasma/BuChE inhibitor, they died immediately after treatment, while wild-type mice treated with the same dose were not affected. Therefore, the role of plasma/BuChE inhibition in neurodevelopment introduces uncertainty as to the long-term effects occurring at doses lower than those inhibiting RBC AChE.

VI.D.2.b. Uncertainties with the Use of AChE Inhibition as an Endpoint for Protecting against Neurodevelopmental Effects

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 CPF may cause neurodevelopmental and neurobehavioral effects in the absence AChE inhibition.

VI.D.2.c. ToxCast™ Profiles and Tox21 HTS Profiles

The ToxCast and Tox21 high-throughput screening assays (HTS) were examined for indications of pathway disruptions that could lead to toxic effects. Zebrafish is a promising test model to examine the potential CPF neurobehavioral effects and compare active concentrations to those inhibiting ChE activity. Abnormal behaviors (increased “fish at rest”, decreased swim speed, decrease in fish with a preference for being on the side or on the edge of their swim lane) occur at CPF exposure levels 10-fold lower than those inhibiting AChE. This provides support for the use of an UF of 10 to account for potential neurodevelopmental effects.

ToxCast and Tox21 provide indications of CPF pathway disruptions in cell adhesion, cell cycle, and cell morphology assays. CPF is also a positive hit for molecular targets that regulate 1) induction and inhibition of CYP enzymes, 2) hormone levels in the brain, 3) endocrine receptor

binding, and 4) steroidogenesis inhibition. However, it is unclear if these impacted pathways are potential noncholinergic key molecular events responsible for the observed CPF neurodevelopmental toxicity *in vivo*.

VI.D.2.d. Animal Studies:

CPF affects several neurotransmitters in the CNS that are critical to behaviors related to mood, emotion, learning, and memory including the endocannabinoids, dopamine, and serotonin. CPF has been shown to affect behavior related to anxiety in animals that is associated with dopamine and serotonin levels. While the overall evidence indicates that CPF may cause neurodevelopmental effects, few *in vivo* animal toxicology studies include doses lower than 1 mg/kg/day, the threshold for ChE inhibition (Carr et al., 2014; Carr et al., 2015a; Carr et al., 2015b, Carr et al., 2017; Mohammed et al, 2015; Silva et al 2017, Gomez–Gimenez et al, 2017; Lee et al, 2015). As such, a definitive conclusion whether these effects are more sensitive than ChE inhibition could not be made at this time. Several in vitro studies have observed negative effects of CPF and CPF-oxon on neuronal growth in tissue culture, including decreased axonal length and inhibition of neurite outgrowth (reviewed in Eaton *et al*., 2008). These in vitro effects occurred at concentrations orders of magnitude less than what would result in AChE inhibition.

VI.D.2.e. Human Studies

Several published reviews have considered the association between prenatal or early pesticide exposure and adverse impacts on human growth and development (Eaton et al., 2008; Prueitt et al., 2011; Goodman et al., 2012; Li et al., 2012; Saunders et al., 2012; Ntzani et al., 2013; Hernández et al., 2016; Furlong et al., 2017). The reviewed studies and those considered in the present assessment may be grouped by type of exposure assessment.

Predicted exposure. Several epidemiology studies used maternal proximity during pregnancy to agricultural pesticide applications to predict exposures or used questionnaires to determine which activities in the participant's past may have led to a potential exposure. Both Harari et al. (2010) and Llop et al. (2013) showed deficits in psychomotor development in children and both evaluated prenatal pesticide use by questionnaire. However, questionnaire responses typically do not provide sufficient information to determine the level of in utero exposure of chlorpyrifos. Berkowitz et al. (2003) found no association between use of pesticides during pregnancy (collected by questionnaire) and the quantitative urinary analysis of OP pesticide biomarkers, underscoring the difficulty of using questionnaires to ascertain exposure. Likewise, the associations reported in studies that relied on pesticide use or application data would have been strengthened by using actual exposure analysis in potential exposed subpopulations.

Measured metabolites. Multiple epidemiology studies utilized urinary metabolites of OP pesticides as biomarkers of exposure. Dialkyl phosphate (DAP) metabolites (DEP, DMP, DETP, DMTP, etc.) are nonspecific metabolites of OP pesticides. Their presence in urine may indicate exposure to an O,O-diethyl pesticide or its degradates, but not a specific active ingredient (Barr and Angerer, 2006). The presence of TCPy in urine also suggests exposure to several different chemicals, including environmental degradates of CPF, CPF-oxon, or CPF-methyl, or TCPy itself. Epidemiological studies have reported associations between total prenatal DAPs, individual DAPs, or TCPy and various decrements in pediatric growth and behavior. However,

when the data were pooled, no consistent dose-effect associations between studies emerged (Engel et al., 2016; Harley et al., 2016). This could have been due to study differences in biomarkers of exposure or effect that limited the ability to cross-compare results. In addition, there is a high degree of within-person variability of urinary biomarkers due to the intermittent nature of exposure, the variety of environmental and dietary sources, individual rates of metabolism and elimination, up-regulation and expression of metabolizing enzymes, the mass balance of the substrates present, as well as substrate binding affinity. Spaan and colleagues (2015) found that when comparing multiple urinary OP metabolites across pregnancy, the within-person variability exceeded the between-person variability. Even while AI-specific information cannot be derived from these metabolites, they can be an indication of the exposure to OPs as a class of pesticides (Barr and Angerer, 2006).

Quantitation of Chlorpyrifos. The only way to unequivocally identify CPF exposure is by measuring the intact pesticide in blood samples. CPF in maternal and cord blood have been associated with various decrements of human growth and development, which are compelling. Blood samples are inherently more difficult to collect then urine. Chlorpyrifos concentrations in blood can be difficult to quantify above the analytical limit of detection (ppt versus ppb levels in urine) (Barr and Angerer, 2006). In addition, the time that the sample was collected (at or within 48 hrs of delivery) is not necessarily indicative of chlorpyrifos exposure during critical windows of in utero development. There currently is no way to precisely categorize CPF exposure throughout pregnancy without highly intrusive and repeated serial sampling of subjects.

Human neurodevelopment is multifactorial. Recent findings indicate a growing association between CPF exposures during gestation and impacts on human growth and development, even though an AOP for chlorpyrifos neurotoxicity has not been elucidated. There may be multiple pathways or covariates independent of AChE inhibition at play, such as PON1-mediated oxidative stress (Harley et al., 2011). In addition, there is evidence that in vitro neuronal growth is impacted by CPF-oxon concentrations below those that inhibit AChE (reviewed in Eaton et al. (2008). There are challenges in incorporating epidemiological results into quantitative risk assessment because of limited exposure data and inconsistencies across studies in dose and effect. However, a lack of a clear mechanism of action does not negate results from numerous observational studies. It is important to consider potential associations documented in epidemiological studies as important mechanistic investigations continue.

VI.D.2.f. The Latest US EPA Methodologies for Deriving PoDs for CPF

US EPA utilized the PBPK portion of the PBPK-PD model from the 2014 US EPA Revised Human Health Risk Assessment to predict CPF blood concentrations in women for comparison with the measured values in the Columbia CCCEH cohort. Subsequently, US EPA revised their risk assessment approach using reverse dosimetry based on a simulated time-weighted average (TWA) concentration of CPF in blood for predicting exposures in adults, infants, and children (US EPA, 2016b). The PoDs were drastically (200-11,000-fold) lower than the PoDs in the US EPA 2014 Revised Human Health Risk Assessment which were based on RBC AChE inhibition. However, for the first approach, SAP did not accept the methodology due to the numerous uncertainties, involved in the design, database uncertainties and missing data. The second approach has not gone through an external scientific review.

As discussed throughout this document, HHA is aware of the uncertainties associated with the use of AChE inhibition as the critical effect for assessing the risk from CPF exposures when potentially more sensitive neurodevelopmental effects have been reported in epidemiology and animal toxicology studies. However, at this time HHA chose not use the PoDs estimated in the Nov 2016 US EPA revised risk assessment. These PoDs were derived using physiologically-based pharmacokinetic modeling to predict time weighted average (TWA) blood concentrations of CPF for the women in the Columbia cohort. HHA carefully reviewed this novel approach and concluded that these PoDs carry substantial uncertainty due to the unknown exposure levels, duration, and critical windows of susceptibility. Because of these uncertainties and the fact that the approach in the 2016 revised risk assessment has not yet undergone external scientific review, HHA has continued to use the 2014 US EPA PoDs based on 10% RBC AChE as the starting point for the present analysis.

VI.D.2.h. Updated Chlorpyrifos PBPK Modeled Steady State (21 Days) Point of Departure (PoD) for Inhalation Exposure for Children 1-2 Years Old

In 2017, Dow AgroSciences LLC (DAS) commented that the steady state (21 day) inhalation PoD of departure for children of 1-2 years old (2.37 mg/m^3) presented in the US EPA 2014 revised chlorpyrifos risk assessment would not achieve a 10% reduction in RBC AChE (Bret *et al.*, 2017). The DAS comment was subsequently confirmed by DPR in communication to US EPA. In a separate analysis requested by DPR, DAS used the DPR default physiological parameters for children 1-2 years old (e.g., 13 kg; Andrews and Patterson, 2000) and estimated an air concentration of 3.0 mg/m^3 that will result in 10% RBC AChE inhibition at 1 hour per day for 21 days (Poet, 2017). Given the fact that HHA adopted all PoD values from the US EPA 2014 risk assessment into the August 2017 DPR draft risk assessment, the updated inhalation PoD value needs to be consistent with the physiological parameters US EPA used for generating other PoD values (e.g., dietary) for children 1-2 years old (e.g., 11 kg rather than 13 kg used previously). Therefore, we estimated a separate 21-day (steady state) PoD value for inhalation using the latest version of the CPF PBPK/PD model (Poet *et al.*, 2017b) and the model input parameters as specified in the US EPA 2014 chlorpyrifos risk assessment. The resulting PoD was 2.85 mg/m^3, which is similar to that generated by DAS but slightly higher than the 2014 US EPA PoD value (Table 62). The simulation result is shown in Figure 13.

Table 62. Comparison of PBPK Modeled 21-Day PoD for Inhalation Exposure of Children (1-2 years old) by US EPA, DAS, and DPR

Inhalation Concentration (mg/m^3)	Exposure Hours per Day for 21 Days	Percent Control RBC AChE Activity	Source
2.37	1	<<10%	US EPA
3.0	1	~10%	DAS
2.85	1	~10%	DPR

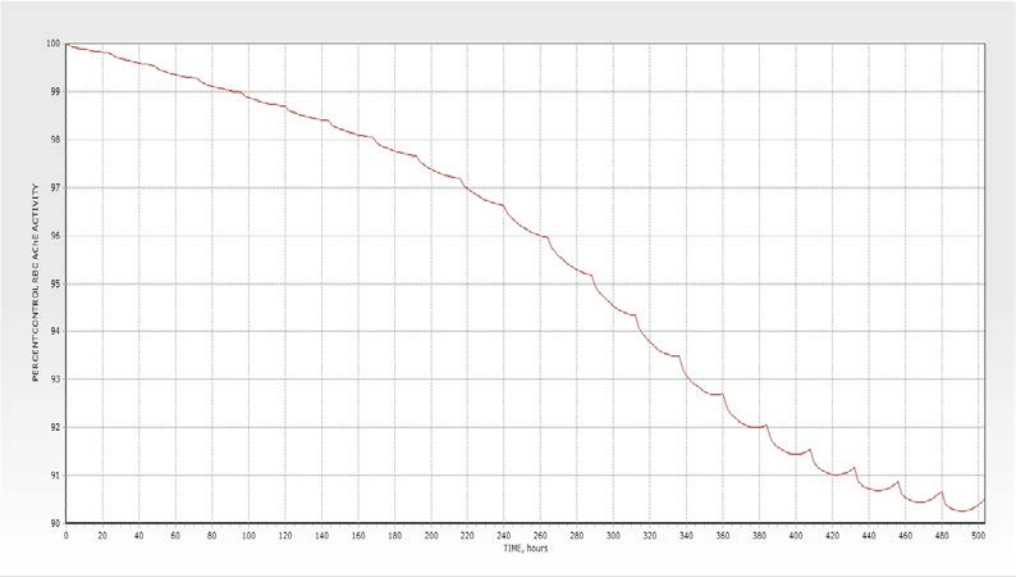

Figure 13. PBPK model simulation result of the percent control RBC AChE activity at an air concentration of 2.85 mg/m3 for one hour per day for 21 days

VI.D.2.h. Risk Assessment Approaches Adopted by Other Regulatory Authorities

Currently, other regulatory authorities employed animal models to derive PoDs for CPF risk assessment. These included European Food Safety Authority (EFSA), the Australian Pesticides and Veterinary Medicines Authority (APVMA), and Health Canada's Pest Management Regulatory Agency (PRMA). Table 63 summarizes the critical endpoints employed by these agencies, all of which are based on 20% ChE inhibition. EFSA and APVMA did not use an additional safety factor for neurodevelopmental effects, whereas Health Canada PMRA applied a UF of 3 for developmental neurotoxicity.

Table 63. Points of Departure, Uncertainty Factors and Reference Doses Generated by Regulatory Agencies

Risk Assessment	**US EPA 2014 Human PBPK-PD** (10% RBC AChEI[a])		**DPR 2015 Human PBPK-PD** (10% RBC AChEI[a])		**EFSA 2014 Rat NOEL** (20% RBC AChEI[b])		**Australia 2017 Human NOEL** (20% RBC or plasma ChE[c])		**Health Canada Rat NOEL** (20% Brain AChEI[d])	
Oral	PoD	RfD	PoD	RfD	PoD	RfD	PoD	RfD	PoD	RfD
Acute	**0.5**	**0.005**	**0.5**	**0.005**	**0.5**	**0.005**	**1**	**0.1**	**0.3**	**0.001**
UF inter **UF intra** **UF FQPA/ neurodev**		1 10 10		1 10 10		10 10 N/A		1 10 N/A		10 10 3
Short term/ chronic	0.08	0.0008	0.08	0.0008	0.1	0.001	0.03	0.003	0.3	0.001
UF inter **UF intra** **UF FQPA/ neurodev**		1 10 10		1 10 10		10 10 N/A		1 10 N/A		10 10 3[d]

a-From US EPA (2014a)
b-European Food Safety Authority (2014) used the adult male rat single dose study (Mendrala and Brzak, 1998) and the comparative cholinesterase study in rat to obtain (Marty and Andrus, 2010) obtain acute and long-term PoDs, respectively.
c-Acute and short-term/chronic PoDs based on a human volunteer study using chlorpyrifos (Coulston et al., 1972)
d=PoDs based on the rat developmental neurotoxicity study (Hoberman, 1998)

CONCLUSION

The focus of the current risk assessment was the rigorous analysis of results from in vivo and in vitro experiments, computational toxicity, epidemiological studies, dietary assessment, pesticide illness reports, and exposure analysis and modeling, to determine the relative risks of exposure to chlorpyrifos to guide risk management decisions.

The database for chlorpyrifos is extensive, covering all aspects of in vitro and in vivo toxicology, metabolism, pharmacokinetics and dynamics. Chlorpyrifos is one of the rare chemicals with a PBPK-PD model which has been extensively peer-reviewed and used in whole or in part by several regulatory bodies. Besides DPR and US EPA, multiple international bodies have conducted human health risk assessments on chlorpyrifos including the European Food Safety Authority (EFSA), the Australian Pesticides and Veterinary Medicines Authority (APVMA), Health Canada's Pest Management Regulatory Agency (PRMA), the Agency for Toxic Substances and Disease Registry (ATSDR), and the Food and Agriculture Organization/ World Health Organization (FAO/WHO). In addition, several epidemiological cohorts, observational studies, and meta analyses have investigated potential associations between adverse human health outcomes and exposure to chlorpyrifos.

The current assessment addresses potential human effects arising from exposure to chlorpyrifos from food, drinking water, air and skin contact, incidental ingestion, as well as aggregate exposures from various combined scenarios. The assessment focused on four at-risk subpopulations: infants (<1 year old), children 1-2 years, children 6-12 years, and women of childbearing age (13-49 years). The critical toxicological points of departure (PoDs) used to characterize the risk from exposure to chlorpyrifos were human equivalent doses estimated by PBPK-PD modeling, adopted from the 2014 US EPA Revised Human Risk Assessment for chlorpyrifos. Risks were calculated as margins of exposure (MOEs), which are equal to the critical PoD divided by the anticipated human exposure level. For this assessment, a MOE of 100 is considered protective of human health for all exposure scenarios. The target of 100 included uncertainty factors (UF) of 1 for interspecies sensitivity, 10 for intraspecies variability, and 10 for potential neurodevelopmental effects. Exposures resulting in MOEs lower than the target of 100 are considered to be of potential health risk to humans. DPR used the PoDs and the target UF of 100 to estimate reference doses or reference concentrations for chlorpyrifos.

No risks were identified from exposures to children and women of childbearing age from dietary sources (food and drinking water) and dermal exposures resulting from spray drift. Potential 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 from combined media (dietary (food only), drinking water, and deposition from spray-drift).

The results of the current assessment found that the aggregate MOEs for a number of combined scenarios were below the target of 100. The air component contributed up to 95% to the aggregate risk. Consequently, the aggregate MOEs were significantly reduced when the air exposure was added to the dermal, non-dietary oral, and dietary exposures. In conclusion, the exposure from air near application sites was identified as the main driver when the aggregate MOEs fell below the target value of 100 for children 1-2 years old.

DPR's Human Health Assessment (HHA) Branch has confidence both in the cholinesterase-based PoDs it employed as toxicological endpoints and in the scenarios it chose to characterize exposure of adults and children, which reflect the typical chlorpyrifos use in California.

The most prominent uncertainties in this assessment include:

1. Reduction of the PoDs by a factor of 10 to address variability within the human population with respect to RBC AChE inhibition. HHA recognizes that the 10-fold default uncertainty factor may not account for the entire range of variability within the human population.

2. Selection of 10% RBC AChE inhibition as the critical toxicity endpoint. This was intended to protect human populations from potential impacts on neurological or neurodevelopmental parameters that are not easily measured and may occur at doses lower than those necessary to elicit AChE inhibition. Since neither the exposure levels of CPF causing neurodevelopmental toxicity nor the critical windows of susceptibility are known, the use of PoDs based on 10% RBC AChE inhibition may not be sufficiently health protective. Consequently, HHA further reduced the PoDs by a factor of 10 to account for the possibility of neurodevelopmental effects.

Although the critical endpoint used in this assessment was 10% RBC AChE inhibition, DPR recognizes that there is a potential for other effects occurring at chlorpyrifos concentrations lower than those that inhibit cholinesterase. There could be other modes of action and adverse outcome pathways leading to neurodevelopmental effects, including non-cholinergic systems, the endocannabinoid system, other signaling pathways, and oxidative stress. At this time, the database does not identify linkage between molecular initiating events, cellular responses, and the developmental neurotoxicity of chlorpyrifos. It is important to note, however, that neurotoxic and neurobehavioral alterations have been documented in experimental animal studies. There is also evidence of potential associations between in utero exposure to chlorpyrifos and altered human growth and behavior later in life. There are acknowledged uncertainties in the human evidence, including a lack of dose-effect relationships, inconsistencies in reported outcomes across studies, and no consistent use of quantitative markers of chlorpyrifos exposure. Nevertheless, human and animal neurodevelopmental effects are compelling.

In conclusion, DPR recognizes that the science is evolving and new data will be analyzed as they become available. The department is confident that this assessment captures the current state of the science of chlorpyrifos toxicity and welcomes comments by the scientific community as we develop approaches to quantitatively address additional adverse outcomes.

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APPENDIX 1.

SUMMARY OF TOXICOLOGY FOR CHLORPYRIFOS

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

DEPARTMENT OF PESTICIDE REGULATION

HUMAN HEALTH ASSESSMENT BRANCH

SUMMARY OF TOXICOLOGY DATA

CHLORPYRIFOS

Chemical Code # 00253 Document Processing Number (DPN) # 0342

SB 950 # 221

Summary initiated: 5/8/86

Revisions on 8/11/86, 11/24/86, 6/5/87, 4/25/89, 11/09/89, 3/16/90, 11/8/90, 5/11/92, 6/28/93, 7/19/94, 9/3/97, 11/13/98, 10/13/99, 9/27/01, 6/5/13, 11/19/13, and June 8, 2015

DATA GAP STATUS

Chronic toxicity, rat: No

data gap, possible adverse effect

Chronic toxicity, dog:

No

data gap, no adverse effect

Oncogenicity, rat:

No

data gap, no adverse effect

Oncogenicity, mouse:

No

data gap, no adverse effect

Reproduction, rat:

No

data gap, no adverse effect

Developmental toxicity, rat: No

data gap, no adverse effect

Developmental toxicity, rabbit: No

data gap, no adverse effect

Gene mutation:

No

data gap, no adverse effect

Chromosome effects:

No

data gap, no adverse effect

DNA damage: No data gap, possible adverse effect

Neurotoxicity: No data gap, no adverse effect

Toxicology one-liners are attached.
All record numbers for the above study types through 284915 (Document No. 342-0969) were examined. This includes all relevant studies indexed by DPR as of June 2, 2015.
In the 1-liners below:
indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: t20150605 chlorpyrifos
Current revision by C. Aldous, June 8, 2015
NOTE: The following symbols may be used in the Table of Contents which follows:
** = data adequately address FIFRA requirement
† = study(ies) flagged as "possible adverse effect"
(N/A) = study type not currently required
This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

METABOLISM AND PHARMACOKINETICS ** (based on collective data)

NOTE: A number of studies in the "Miscellaneous" section near the end of this Summary include metabolism, pharmacokinetics, and cholinesterase inhibition data.

342-0343 071390 Nolan, R. J., M. D. Dryzga, B. D. Landenberger, and P. E. Kastl, "Chlorpyrifos: tissue distribution and metabolism of orally administered ^{14}C-labeled chlorpyrifos in Fischer 344 rats," The Dow Chemical Company, Midland, MI, 12/23/87. Laboratory Study # K-044793-(76). Five rats/sex/group were dosed by gavage in 2 ml/kg corn oil in single labeled doses of 0.5 or 25 mg/kg or 15 consecutive daily doses of unlabeled chlorpyrifos at 0.5 mg/kg/d, followed 1 day after the 15th dose with a single labeled dose of 0.5 mg/kg. Labeled chlorpyrifos (>99% radiopurity) was 12 µCi per gram of corn oil regardless of dose. Only the 3,5,6-trichloro-2-pyridinol group was labeled. Unlabeled chlorpyrifos, used to dilute the high dose group, was 99.9% purity. Investigators evaluated label in urine, feces, and tissues, and identified the three significant urinary metabolites. Urine plus cage wash accounted for 86 to 93% of administered label, regardless of sex or dosing regimen. Six to 11% of label was found in feces. Urinary excretion was rapid: usually over 50% of administered dose was collected in urine within the first 12 hours (T1/2 was 8-9 hours for single or multiple 0.5 mg/kg treatments, and somewhat longer for 25 mg/kg rats). Urinary metabolites were composed chiefly of 3,5,6-trichloro-2-pyridinol, and usually slightly more of its glucuronide, collectively accounting for over 90% of urinary metabolites. About 5% of urinary residues consisted of the sulfate conjugate of 3,5,6-trichloro-2-pyridinol. Parent chlorpyrifos was not found in urine. Most fecal label was obtained

within the first 24 hours. Exhaled CO2 was trapped for radioanalysis from the 25 mg/kg group. This collection accounted for <0.01% of administered dose. Fecal metabolites were not assessed. Tissue residues were assessed at 72 hrs (M) and at 144 hrs (F). Total tissue residues were very small (0.2% of administered dose in 25 mg/kg group) to negligible (<0.01%), and generally only quantifiable in peri-renal fat (M and F). In the 25 mg/kg groups only, tiny but quantifiable residues were also found in liver (M) and ovaries. This is a valid supplementary study. Aldous, June 5, 2015.

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat **

**342-716; 154442; Stebbins, K. E., "Dursban F Insecticidal Chemical: Acute Oral Toxicity Study in Fischer 344 rats," study type 811; The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044793-102A; 11/27/96; Dursban F Insecticidal Chemical (purity: 97.6%); 5 animals/sex/group; Doses: 50, 100, 500 mg/kg as 3% suspension in 0.5% aqueous solution of Methocel A4M; Mortality: 50 (M/F:0/5), 100 (M/F:0/5), 500 (M/F:5/5), deaths occurring with 3 days after dosing; Clinical Observations: fecal soiling, lacrimation, urine soiling, salivation, decreased activity; Necropsy: no treatment-related lesions noted; LD50 (M/F): 223 mg/kg; Toxicity Category II; Study acceptable. (Moore, 5/29/97)

**342-708; 154314; Nissimov, S. and A. Nyska, "Pyrinex Tech.: Acute Oral Toxicity in the rat," study type 811; Life Science Research Israel Ltd., Ness Ziona 70451, Israel; Study No. MAK/056/PYR; 5/12/84; Pyrinex Tech; 5 animals/sex/group; Doses: 90, 164, 298, 543, 987 mg/kg, in corn oil; Mortality: 90 (M/F:0/5), 164 (M:0/5, F:4/5), 298 (M/F:5/5), 543 (M/F:5/5), 987 (M/F:5/5); Clinical Observations: tremors, hunched posture, salivation, diarrhea, decreased motor activity, ataxia; Necropsy: hemorrhagic and/or ulcerated stomach and intestines; LD50 (95% confidence interval): (M) 221 (181 to 269) mg/kg, (F) 144 (105 to 200) mg/kg; Toxicity Category II; Study acceptable. (Moore, 6/10/97)

Acute dermal toxicity **

**342-716; 154444; Stebbins, K. E., "Dursban F Insecticidal Chemical: Acute Dermal Toxicity Study in New Zealand White Rabbits," study type 812; The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044793-102D; 11/27/96; Dursban F Insecticidal Chemical (purity: 97.6%); 5 animals/sex/group; Doses: 2000, 5000 mg/kg, test material liquefied prior to application, 24 hour exposure; No mortality; Clinical Observations: fecal soiling, dermal irritation at the site of application; Necropsy: no treatment-related lesions; LD50 (M/F) > 5000 mg/kg; Toxicity Category IV; Study acceptable. (Moore, 5/30/97)

**342-709; 154315; Nissimov, S. and A. Nyska, "Pyrinex Tech.: Acute Dermal Toxicity in rabbits," study type 812; Life Science Research Israel Ltd., Ness Ziona 70451, Israel; Study No. MAK/059/PYR; 5/12/84; Pyrinex Tech; 5 animals/sex; Dose: 2000 mg/kg, liquefied prior to application, 24 hour exposure, semi-occlusive wrap; No mortality; Clinical Observations: no

treatment-related signs; Necropsy: congested lungs, skin lesions, multiple petechiae on thymus; LD50 (M/F) > 2000 mg/kg; Toxicity Category III; Study acceptable. (Moore, 6/10/97)

Acute inhalation toxicity, rat **

**342-710; 154316; Buch, S. A., "Pyrinex Tech.: Acute Inhalation Toxicity in rats," study type 813; Life Science Research, Stock, Essex, England; Study No. 80/MAK025/362; 8/27/80; Pyrinex Tech (purity: 95.%); 5 animals/sex/group unless otherwise noted; Exposure Concentrations (gravimetric): 1.69 (F only), 2.23, 2.98, 3.56, 4.07 mg/l, MMAD (GSD): 7.4 (2.2), 7.9 (1.7), 8.2 (1.9), 8.0 (2.0), 8.6 (2.1) μm, respectively, respirable concentration (mass of particles < 10 μm): 1.40, 1.86, 2.61, 3.01, 3.47 mg/l, respectively, 4 hour nose-only exposure (test material was prepared as a 60% (w/v) in xylene) (concentrations based upon non-volatile portion of exposure atmosphere); Mortality: 1.69 (F:1/5), 2.23 (M:0/5, F:2/5), 2.98 (M:0/5, F:3/5), 3.56 (M:0/5, F:2/5), 4.07 (M:0/10, F:4/5); Clinical Observations: decreased motor activity, hunched posture, ataxia, tremor, hypothermia, piloerection, pigmented stain around eye and snout, gasping, bradypnea, muscle fasciculations; Necropsy: lungs pale and/or congested, liver pale with accentuation of lobular pattern, increased relative lung weights among the decedents; LC50 (95% confidence limit): (M) > 4.07 mg/l, (F) 2.89 (2.01 to 4.16) mg/l; Toxicity Category III; Study acceptable. (Moore, 6/11/97)

342-343; 71387; Landry, T. D., D. A. Dittenber, L. G. Lomax, and J. J. Momany-Pfruender, "Chlorpyrifos: an acute vapor inhalation toxicity study with Fischer 344 rats," study type 813; Dow Chemical Company, Mammalian and Environmental Toxicology Research Laboratory, Midland MI; Lab Study No. K-44793-74; 12/3/86; Chlorpyrifos (Reference No. AGR 219646; purity = 100%), used neat; 0 (air) (24M/24F), 3.5 (6M/6F), 6 (12M/12F), 14 (6M/6F) ppm (analytical); vapor inhalation, 6-hour, whole-body and nose-only exposures; Mortality- one male at 6 ppm (attributed to physical trauma); Clinical Observations- reduced plasma cholinesterase activity (13-24% reduction) in 6 ppm group only (attributed to oral ingestion or dermal absorption of the dose); hyperactivity (considered not exposure-related); Necropsy- no treatment-related findings; reported LC50 (M and F) > 14 ppm (0.22 mg/l); Supplemental. (Duncan, 6/21/91)

Primary eye irritation, rabbit **

**342-716; 154445; Stebbins, K. E., "Dursban F Insecticidal Chemical: Primary Eye Irritation Study in New Zealand White Rabbits," study type 814, The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044793-102C; 11/27/96; Dursban F Insecticidal Chemical (purity:97.6%); 6 animals; Dose: 0.1 ml/eye, liquefied prior to application; Observations: no ocular irritation evident at 24 hours; Toxicity Category IV; Study acceptable. (Moore, 5/30/97)

**342-711; 154317; Buch, S. A. and J. R. Gardner, "Pyrinex Tech.: Irritance to rabbit eye," study type 814; Life Science Research, Stock, Essex, England; Study No. 80/MAK023/143; 4/30/80; Pyrinex Tech; 6 animals (eyes not rinsed); Dose: 100 mg/eye; Observations: no corneal opacity nor iritis evident, Conjunctiva (redness)-grades 2 (1/6) and 1 (5/6) at 24 hours, grade 1 (1/6) through 7 days (termination), no chemosis nor discharge evident at 24 hours; Toxicity Category III; Study acceptable. (Moore, 6/11/97)

Primary dermal irritation **

**342-716; 154446; Stebbins, K. E., "Dursban F Insecticidal Chemical: Primary Dermal Irritation Study in New Zealand White Rabbits," study type 815; The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044973-102B; 11/27/96; Dursban F Insecticidal Chemical (purity: 97.6%); 6 animals; Dose: 0.5 ml/site, liquefied prior to application, 4 hour exposure; Observations: erythema-grade 1 (6/6) at 30 minutes post-exposure, grade 1 (4/6) at 24 hours, grade 1 (2/6) at 48 and 72 hours, clear by 7 days; Toxicity Category IV; Study acceptable. (Moore, 5/30/97)

**342-712; 154319; Buch, S. A. and J. R. Gardner, "Pyrinex Tech.: Irritance to rabbit skin," study type 815; Life Science Research, Stock, Essex, England; Study No. 80/MAK024/144; 4/30/80; Pyrinex Tech; 6 animals; Dose: 0.5 gm/site (4 sites, 2 intact, 2 abraded), moistened with 0.2 ml of physiological saline, 23 hour exposure, occlusive wrap; Observations: (intact sites) erythema-grades 2 (3/6) and 1 (3/6) at 24 hours post-dosing, grade 1 (1/6) at 72 hours and on day 8, edema-grade 1 (1/6) at 24 hours post-dosing, clear by 72 hours; Toxicity Category IV; Study acceptable. (Moore, 6/11/97)

Dermal sensitization **

342-0716 154447 Stebbins, K. E., "Dursban F Insecticidal Chemical: Dermal Sensitization Potential in Hartley Albino Guinea Pigs," The Dow Chemical Company, Midland, MI, 11/27/96. Laboratory Study # K-044793-102E. Investigators first determined that the lowest non-irritating dose of Dursban F was 1% in dipropylene glycol monomethyl ether (DPGME). This dose level was used in the primary study. In all sensitization cases, induction was performed weekly for 3 weeks, and challenge followed two weeks after the third induction (with skin site examination 24 and 48 hrs after challenge). On each occasion, 0.4 ml of material was applied to clipped, intact skin for 6 hours. Test materials for positive controls was either DER 331 epoxy resin (neat) and dinitrochlorobenzene (DNCB, 0.5% in DPGME vehicle). Groups of five naïve animals were dosed twice (one week apart) with each of the three treatments as non-induced controls. Under these circumstances, Dursban F induction/challenge group showed erythema in only one animal (the same animal showing "slight" erythema during induction week 1 and again "slight" erythema 48 hrs after challenge). Main study positive controls were uniformly negative for skin irritation during the first two induction treatments, then frequently showed "slight" erythema at the third induction treatment. Both positive controls typically displayed "slight" to "moderate" erythema at challenge. Treatments of naïve animals were uniformly negative, except for one Dursban F animal with "slight" erythema. Thus test system was viable, and **negative for dermal sensitization for Dursban F. Study is **acceptable**, with no adverse effects. Aldous, 4/14/15.

342-0713 154320 Berman, C. L., "Evaluation of Chlorpyrifos (Pyrinex) for dermal sensitization of guinea pig," Arthur D. Little, Inc., Cambridge, MA, 10/21/1987. Test article was chlorpyrifos, 96.8% purity, Technical grade. This study was examined on 7/29/97 by C. Rech of DPR, who noted several deficiencies, and requested a replacement study. This unacceptable study did not indicate sensitization potential. (Aldous, June 3, 2015).

**342-0744 162453 Bassett, J. and M. Watson, "Dermal Sensitization study (closed-patch repeated insult) in guinea pigs with Chlorpyrifos Technical (Pyrinex)," Department of

Toxicology, Ricerca, Inc., Painesville, OH, 3/31/98. Technical chlorpyrifos (97% purity) was administered to 20 Hartley guinea pigs for the induction phase at 50% concentration in peanut oil, 0.4 ml/site, administered to the shaved dorsal and lateral skin 3 times at weekly intervals. Challenge was 2 weeks after the last induction exposure, administered in 50% propylene glycol. Chlorpyrifos did not elicit a challenge response (i.e. is not a sensitizer). Positive control (DCNB) was effective. This study was considered as negative for sensitization and acceptable by DPR reviewers, D. E. Haskell and J. R. Sanborn (review of Dec. 2, 1998).

SUBCHRONIC STUDIES

Subchronic Oral toxicity, rat:

342-354 74494 Szabo, J. R., J. T. Young, and M. Grandjean, "Chlorpyrifos: 13-week dietary toxicity study in Fischer - 344 rats." Lake Jackson Research Center [The Dow Chemical Co.], Freeport, Texas, 12/28/88. This study was submitted by Dow to contest the CDFA decision of a cholinesterase (ChE) NOEL at 0.05 mg/kg/d in the 2-year study, 345:072300. No comprehensive CDFA review of this subchronic study is necessary at this time, since the purpose of the 13-week study was to set dose levels for the cited 2-year study, which has already been accepted by CDFA. This subchronic study found statistically reduced plasma ChE levels ($p < 0.05$, two tailed) at day 44, but not at day 91. Investigators concluded findings at day 44 "not considered to be of toxicologic or biologic significance." CDFA concludes that the findings are probably treatment effects, which however have no apparent toxicological consequence: the plasma ChE NOEL remains 0.05 mg/kg/d, but a practical NOAEL for ChE inhibition is 0.1 mg/kg/d. C. Aldous, 11/9/89.

Subchronic Oral toxicity, non-rodent: (a supplementary 3-mo. dog study has been reviewed. No further non-rodent subchronic data are requested at this time.

342-306 063996 [Author appears to be McCollister, S. B.], "Results of 93-day dietary feeding studies of O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate in beagle hounds," 1/15/64. This study pre-dates modern guidelines, and should be considered only for information on major symptoms of toxicity. Dogs were initially administered chlorpyrifos (98% purity) at 0, 200, 600, or 2000 ppm (report designates units of initial exposure as 0, 0.02, 0.06, and 0.2 percent in diet). There were 4 controls/sex, and 2/sex for each of the other groups. None of these treated dose levels were sustainable, due to cholinergic symptoms such as "dilated and watery eyes, loose stools, vomiting, rough coats, labored breathing and tremors of the legs and head." The 2000 ppm dogs were "essentially starving" as of treatment day 5, so that their diet was reduced to 0.006% (60 ppm) for the balance of the study. The dogs administered initially 600 ppm "were developing gross cholinergic symptoms," and had diets reduced to 0.002% (20 ppm) after 16 days. Dogs originally administered 200 ppm were placed on control diet from day 45 onward. An additional group (N = 2/sex) was administered 200 ppm chlorpyrifos for about 45 days prior to sacrifice (designated as "Group B," with estimated mean exposure of 3.4 mg/kg/d). Dogs were evaluated periodically for plasma and RBC cholinesterase (ChE), and brain acetylcholinesterase (AChE) was assessed at termination. Hematology, limited clinical chemistry, and terminal necropsy and histopathology were also recorded. These data were initially reviewed mainly to justify dose levels used in the chronic dog study (Record No. 036338). Small group sizes and altered dosing regimens limited the utility of this study. Group

B 200 ppm dogs lost weight during their 45-day treatment, at a life stage when control dogs were still gaining weight. In particular one of the two Group B females lost 1.4 kg, and the other (which died shortly before scheduled sacrifice) lost 1.65 kg. The two Group B dogs surviving to termination and which had brain tissue assayed for AChE had brain AChE activities of about 50% of controls. The most relevant blood ChE data for these dogs was at 27 days of continuous treatment: at this time, the highly variable plasma ChE averaged about 10% of pre-exposure activity, and similarly variable RBC AChE activity was less than 20% of pre-exposure activity. Group A 200 ppm dogs had progressively diminishing plasma and RBC AChE inhibition over the time frame from 14 to 41 days of continuous exposure. When these dogs came off treatment, plasma ChE activity was visibly improving by 3 days, and was roughly 80% of pre-treatment levels by the 18th day off treatment. RBC AChE activity was slower to recover: with about 50% of pre-dosing activity between recovery days 18 and 32. RBC AChE activity was still below baseline at the last blood assay on recovery day 41. Brain AChE in these Group A 200 ppm dogs appeared to be in the normal range after 48 days of recovery. Dogs administered the medium dose (60 ppm for all but the first 5 study days) finished the study with plasma and RBC AChE activities at about 50% of pre-exposure values. At termination, males had brain AChE activity in the normal range, whereas females had implausibly low brain activities (i.e. lower than those observed in 200 ppm dogs after about 45 days of dosing). Dogs on the lowest sustained dose level (20 ppm) had plasma ChE activities of about 25% of pre-treatment levels, and RBC AChE activities of about 50% of pre-treatment levels. The 20 ppm males had normal brain AChE activity at termination, whereas one female had normal brain AChE activity, and one had about 40% of normal brain activity. In summary, although this study does not meet modern guidelines, had small group sizes and large variability in key responses, responses provide useful information on high dose effects to augment results from the later dog chronic studies. "One-liner" was re-written by Aldous on June 4, 2015 in support of risk assessment efforts in DPR.

Subchronic Inhalation toxicity, rat:

342-0967 284609 Newton, P. E., "A thirteen week nose-only inhalation toxicity study of chlorpyrifos technical (Pyrinex) in the rat," Bio/dynamics Inc., East Millstone, NJ, 11/14/88, Project No. 88-8058. Fifteen F344 rats/sex/group were dosed by nose-only inhalation to chlorpyrifos vapors (Pyrinex Technical, 95% purity) at targeted concentrations of 0, 5, 10, and 20 ppb, respectively [6 hours/day, 5 days/week, for 13 weeks]. There were no treatment effects on clinical signs (in chamber or at detailed weekly examinations), or on body weight, food consumption, hematology, clinical chemistry [other than possible plasma cholinesterase (ChE)]. Ophthalmology, necropsy observations, and histopathology findings were negative. Brain and RBC AChE activities were unaffected. The 20 ppb male plasma ChE activities were lower than any other contemporary groups and also lower than the limited pre-test ChE activities available. This reviewer considers that this represents a plausible treatment effect, with a NOEL of 10 ppb. NOEL for females = 20 ppb (no changes observed). This is a valid supplementary study (not a study design routinely expected under FIFRA requirements). See also the 1986 study: 342-0343 071389 (Corley et al.), which did **not** find any ChE effects at similar dose levels in nose-only vapor subchronic inhalation conditions like the present study. These equivocal, marginal plasma ChE findings are not designated as "possible adverse effects" under these circumstances. Aldous, June 3, 2015.

342-0967 284608. This is a brief report of corrections to 342-0967 284609, above. The cause of death had been erroneously coded for two rats in the original report. Survival was not dose-related in this study, and the corrections had no consequential impact on study interpretation.

Dermal toxicity, 21/28-day or 90-day:

342-0343 071391 Calhoun, L. L. and K. A. Johnson, "4-day dermal probe and 21-day dermal toxicity studies in Fischer 344 rats," The Dow Chemical Company, Midland, MI, Sept. 1, 1988. Laboratory Study Nos. K-044793-085, K-044793-086. Chlorpyrifos, purity 100±0.1%, was applied in corn oil vehicle 6 hours/treatment to intact clipped dorsal skin (under gauze, secured by bandages) as indicated. Four female rats/sex/group were dosed by dermal application in corn oil at 0, 1, 10, 100, or 500 mg/kg/d for 4 consecutive days at 6 hours/treatment in a **probe study**. That study found that plasma cholinesterase was inhibited by 45%, 91%, and 97% at 10, 100, and 500 mg/kg/d, respectively. Also, RBC cholinesterase was inhibited by 16%, 49%, and 75% at respective dose levels. There were no other definitive findings in the probe study (which also assessed application site response, clinical signs, and body weight). The **primary** study was a 21-day dermal regimen, with dosing each weekday for a total of 15 exposures at 0, 0.1, 0.5, 1, or 5 mg/kg/d (N = 5/sex). Necropsy followed 2 consecutive treatment days in the final week. Investigators evaluated the parameters of the pilot study, plus a limited FOB, hematology, clinical chemistry, and histopathology. There were no definitive treatment effects in the primary study, hence the highest dose tested of 5 mg/kg/d is the NOEL for both sexes. This study is supplementary and not upgradeable (mainly because the dose range in the primary study was well below what the probe study showed to be supportable). Aldous, June 5, 2015.

CHRONIC STUDIES

Combined (chronic/oncogenicity), rat ** † ("possible adverse effect" based on non-oncogenicity findings in Record No. 153114, rat oncogenicity study)

**342-345 072300 Young, J. T., and M. Grandjean, "Chlorpyrifos: 2-year dietary chronic toxicity-oncogenicity study in Fischer-344 rats". Dow Chemical Co., Freeport TX, 12/23/88. Chlorpyrifos ("AGR 214637"), 98.5%, in diet at 0, 0.05, 0.1, 1, and 10 mg/kg/d. 10/sex/dose designated for 1-year interim sacrifice: 50/sex/dose designated for 2-year duration. Cholinesterase (ChE) inhibition NOEL = 0.05 mg/kg/d (based on slight plasma ChE inhibition at 0.1 mg/kg/d in females). Acetylcholinesterase ChE inhibition NOAEL of 0.1 mg/kg/d is nevertheless supportable, considering the issues discussed in the review for 354:074494. The NOEL for effects other than ChE inhibition was 0.1 mg/kg/d [based on very slight ($\leq$ 3%) but often statistically significant body weight decrease in 1 mg/kg/d males]. Body weights were statistically significantly reduced in 10 mg/kg/d males (7 to 9% throughout study). The "non-ChE effects" NOAEL was 1 mg/kg/d. Findings at 10 mg/kg/d were frequent perineal yellow staining in females, approximately 50% brain ChE inhibition in males and females, a slight increase in the degree of vacuolation of the adrenal zona fasciculata (males only), and a slight increase in diffuse retinal degeneration in 10 mg/kg/d females. None of these findings indicates possible adverse health effects (see review). ACCEPTABLE. C. Aldous, 4/21/89, 11/9/89 (see 354:074494). NOTE: Another rat study (see Record No. 153114 under AOncogenicity, Rat@ similarly identified retinal atrophy and cataracts at the highest dose tested (100 ppm in the latter case).

342-363 087917 (supplemental information to 342-345:072300). "Macroscopic postmortem examination of the eyes and associated structures in albino rats (Dow Method)". (Refers to technique used at Freeport, TX, facility), method description dated 9/11/89. Methodology was presented in accordance with a CDFA request, which was made in the 4/21/89 CDFA review of the cited study. C. Aldous, 3/16/90.

342-250 and -251 036335-036337 McCollister, S. B., R. J. Kociba, P. J. Gehring, and C. G. Humiston, "Results of Two-Year Dietary Feeding Studies on DOWCO 179 in Rats" Dow Chemical, Midland, Michigan, 9/20/71. Chlorpyrifos, (presumed technical); 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/d in diet. NOEL cholinesterase enzyme inhibition = 0.1 mg/kg/d. NOEL for other systemic effects = 3.0 mg/kg/d (HDT). No oncogenicity observed. Incomplete, UNACCEPTABLE, and not upgradeable Too few animals, too much attrition due to disease (largely chronic murine pneumonia) & dose levels not justified and apparently below the MTD. C. Aldous, 1/28/86.

EPA 1-liner: [2-year feeding, rat, Dow Chemical Co, 9/20/71] Systemic NOEL 3.0 mg/kg/d (HDT); ChE NOEL = 0.1 mg/kg/d. Carcinogenic potential negative up to 3.0 mg/kg/d (HDT). Core grade, Supplementary.

342-044 031074 Published summary of 250/251:036335-036337.

342-013/053 031070 Summary of 250/251:036335-036337.

EPA 1-liner: [2-year feeding, rat, Dow Chemical Co, 9/20/71] Systemic NOEL 3.0 mg/kg/d (HDT); ChE NOEL = 0.1 mg/kg/d. Carcinogenic potential negative up to 3.0 mg/kg/d (HDT). Core grade, Supplementary.

342-044 031074 Published summary of 250/251:036335-036337.

342-013/053 031070 Summary of 250/251:036335-036337.

Chronic, dog **

**342-0252 036338-036339 McCollister, S. B., R. J. Kociba, P. J. Gehring, and C. G. Humiston, "Results of Two-Year Dietary Feeding Studies on DOWCO® 179 in Beagle Dogs," Dow Chemical, Midland, MI, 12/10/71. Chlorpyrifos (97.2% purity) was administered in diets at concentrations adjusted to provide 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/d. This study had two phases. In Phase A, there were 3/sex/group treated for 1 year, at which time 1/sex was necropsied. The remaining 2/sex were taken off treatment for 3 months prior to necropsy to evaluate recovery. In Phase B, 4/sex were dosed for 2 years at the above levels. Investigators assessed standard parameters of chronic studies. To assess cholinesterase (ChE) effects, plasma and RBC AChE activities were assayed 3 times pre-treatment and at 6 intervals during Phase A treatment. In Phase B, plasma and RBC AChE activities were assayed twice pre-treatment and at 8 intervals during treatment. Brain ChE was assessed at sacrifices of all dogs in both phases. Plasma ChE inhibition NOEL = 0.01 ppm, based on dose-related inhibition at 0.03 ppm and above. RBC AChE NOEL = 0.1 ppm, based on strong inhibition at 1.0 and 3.0 ppm compared to

the same subjects at pre-treatment assessments. (See also Record No. 284915, which is a composite analysis of the RBC data from this study). Brain ChE activity at 3.0 mg/kg/d was reduced by an average of about 18%, with no evident sex difference in magnitude of response. There is a NOEL of 1.0 mg/kg/d for brain ChE. The NOEL for other effects, including behavioral observations, was the highest dose tested of 3.0 mg/kg/d. The study was designated as **acceptable** on 3/16/90, on receipt of details on preparation of treated food. Previous objections of CDFA to this study were (1) concerns that dosage range may not have adequately challenged the dogs, and (2) lack of reporting of ophthalmological examination data in the final report. These were addressed in submissions 306:063996 and 338:070883, respectively. This study was examined by C. Aldous on1/29/86, 4/11/89, 3/16/90 (see also rebuttal response of 6/4/87 and minutes of meeting with Dow Chemical Co. representatives on 6/29/88). A final examination by Aldous on June 3, 2015 updated this summary and noted recent submission of the cited Record No. 284915 data. This study does not indicate an "adverse effect." ChE enzyme responses in this study are well-characterized and consistent with results of other rat dietary studies such as the rat subchronic, developmental toxicity, and reproductive effects studies.

342-363 087918 (Addendum to 342-252:036338, combined dog study). Submission contains mean body weights/sex and average food consumption for a 6-week period. At the end of the 6-week period, it was determined that 100 ppm in diet corresponded closely to 3.0 mg/kg/d in either sex. From that time on, diets were prepared at fixed levels of 100, 33, 3.3, 1.0, and 0.33 ppm by serial dilutions of diets. These data permit an upgrade of the 1971 dog study to ACCEPTABLE status. Aldous, 3/16/90.

342-0969 270309 (Supplementary to Document No. 342-0252, Record Nos. 036338-036339), Authors of the re-analysis are Mattsson, J. L., L. Holden, D. L. Eisenbrandt, and J. E. Gibson. "Reanalysis with optimized power of red blood cell acetylcholinesterase activity from a 1-year dietary treatment of dogs to chlorpyrifos." The date of the re-analysis was 9/22/2000. Study ID: GHC-5127. Chlorpyrifos (97.2% purity) in the dog chronic study was administered in diets at concentrations adjusted to provide 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/d. That study had two phases at the above dose levels, which were comparable in design, so that parallel results could properly be considered together. The present analysis was confined to RBC acetylcholinesterase (AChE) inhibition analysis. Four figures show RBC AChE activities by phase and sex consistent with tabular summary data in Record No. 036338. These figures show marked inhibition of RBC AChE activity at 1.0 and 3.0 mg/kg/d, whereas AChE activities of other groups tended to cluster together at any given time point. Individual pre-treatment AChE activities had more influence on subsequent treatment-phase activities than did possible treatment group effects, except at the two highest dose levels. When investigators normalized the baseline for each group pre-treatment mean, combining data for both sexes in both phases at assay intervals during the first year gave N = 14. A depiction of inter-group differences on this basis found no meaningful differences between control and treatment groups through 0.1 mg/kg/d. When all assays during the first year of treatment were considered together for each group, activity of the 1.0 mg/kg/d group was nearly 50% below baseline, and the 3.0 mg/kg/d group activity was 80% below baseline, whereas all other groups remained within about 4% of baseline. Collectively, these amalgamated data support a NOEL of 0.1 mg/kg/d for RBC AChE. Aldous, June 2, 2015.

342-273 056902 (Tab 3) EPA Office of Pesticide Programs, Toxicology Branch review of study 252:036338-036339. The review was submitted on Oct. 10, 1985 as OPP Toxicology Branch Document #004712. The review classified the study as "Core Minimum Data".

EPA 1-liner: [2-year feeding - dog; Dow Chem. Co.; 12/10/71] Systemic NOEL = > 3.0 mg/kg/d (HDT); Plasma ChE NOEL = 0.01 mg/kg/d; Plasma ChE LEL = 0.10 mg/kg; RBC AChE NOEL = 0.10 mg/kg/d; RBC AChE LEL = 1.0 mg/kg; Brain ChE NOEL = 1.0 mg/kg/d; Brain ChE LEL = 3 mg/kg; Core grade, supplementary [note upgrade to "core minimum" status, indicated in 273:042783].

342-338 070881-070882 are dietary analyses and analytical methods descriptions. These data were evaluated with respect to study 252:036338 in the 4/11/89 CDFA review.

342-338 070883 is a supplement to the original 2-year dog feeding study report. Supplement included ophthalmology data. These data had been submitted to EPA in 1985. These data were evaluated with respect to study 252:036338 in the 4/11/89 CDFA review.

342-044 031073 Published summary of 252:036338.

342-013/053 031070 Summaries of 252:036338-36339

Oncogenicity, rat (see "Combined, Rat" above)

****342-692 153114** Crown, S., "Pyrinex technical oncogenicity study in the rat", Life Science Research Israel, Ltd., July 12, 1990. Laboratory Study # MAK/095/PYR. Pyrinex (chlorpyrifos), 96.1% purity, was administered in diet to 60 F344 rats/sex/group at 0.2, 5, and 100 ppm. There were two control groups (with and without corn oil mixing supplement), each composed of 60/sex/group. Treatment was for 2 yr, except that 5/sex/group were sacrificed at wk 50 for brain cholinesterase (ChE) assays. ChE enzyme inhibition NOEL = 0.2 ppm (inhibition of plasma ChE at 5 ppm). NOEL for non-ChE-related changes = 5 ppm. No definitive cholinergic signs were evident at any dose level. Findings at 100 ppm included modest body weight decrements and over 50% brain ChE inhibition in both sexes, and an increase over baseline incidences of diffuse retinal atrophy and cataracts in 100 ppm females. The latter findings are **"possible adverse effects**" in an **acceptable** oncogenicity study. Aldous, 8/28/97.

Oncogenicity, mouse **

**342-693 153115 Gur, E., "Pyrinex technical oncogenicity study in the mouse", Life Science Research Israel, Ltd.,10/15/92. Laboratory Study # MAK/106/PYR. Fifty-nine CD-1 mice/sex/group were dosed for 79 weeks with Pyrinex technical (chlorpyrifos) in diet at 0, 5, 50, or 250 ppm. An additional 5/sex/group were killed at week 42 for cholinesterase (ChE) evaluation. There was no ChE NOEL in the tested dosage range (dose-related inhibition of plasma ChE in both sexes at weeks 42 and 78). Brain ChE was modestly reduced at 50 ppm and greatly reduced at 250 ppm (residual activity about 20% or less in both sexes and both sampling intervals). RBC AChE was reduced at 250 ppm only. There were no definitive cholinergic signs at any dose. NOEL for other effects was 5 ppm (males displayed excessive lacrimation, opaque

eyes, and hair loss around eyes: all plausibly related to contact irritability of test article with resultant scratching). High dose findings, in addition to signs consistent with local irritation, included hepatocyte vacuolation and cystic dilatation of bulbourethral glands (males), and alveolar macrophage accumulation in lungs (females). Male body weights and food consumption were decreased at 250 ppm, and water consumption was sharply reduced in both sexes at that dose level. Survival of high dose males was remarkably higher than other groups. This is an **acceptable** oncogenicity study with no adverse chronic effects. Aldous, 8/22/97.

**342-253 036340 Warner, S. D., C. G. Gerbig, R. J. Strebing, and J. A. Molello, "Results of a two-year toxicity and oncogenic study of Chlorpyrifos administered to CD-1 mice in the diet," Dow Chemical Toxicology Laboratory, Indianapolis, Indiana, 3/4/80. Chlorpyrifos, Ref. No. 1-500-2: 99.6% purity at 0, 0.5, 5.0, and 15.0 ppm in diet. NOEL = 15 ppm (no toxicity). No oncogenicity. ACCEPTABLE, based on re-reading of blood smears by S. D. Warner, D.V.M., PhD (data in CDFA record 315:065762) answering a question by CDFA regarding possible effects on lymphocytes, (see 5/29/87 CDFA review). (Other concerns which CDFA had on this report were addressed in the 5/29/87 CDFA review). C. Aldous, 1/31/86, 5/29/87, 4/12/89.

342-273 042782 (Tab #4) Supplemental to 253:36340. Davies, D. B., J. T. Tollett, and L. G. Lomax, "Chlorpyrifos: A Four -Week Dietary Study in CD-1 Mice," Dow Chemical, Midland, MI. Dietary administration of 0 or 15 ppm chlorpyrifos (95.7% purity) to CD-1 mice. 4 week study with body weights slightly reduced and plasma and serum ChE levels statistically significantly reduced (see especially. Table 13). This study supports dose level selection for the oncogenicity study (such as 253:036340, above). After 4 weeks, treated mice had about 10% of control plasma cholinesterase (ChE) activity, and about 50% of RBC AChE activity. Brain AChE activity was statistically reduced in treated females and statistically elevated in treated males: magnitudes were small in both cases and appear to have been incidental. Examined 11/24/86 and again on 6/4/15 by C. Aldous. No written review was required or performed.

EPA 1-liner: [2-Year oncogenic - mice; Dow Chemical Co.; 3/04/80]: Systemic and oncogenic NOEL > 15 ppm (HDT). Core grade, minimum.

342-290:050623 (Rebuttal/Additional data to 253:36340) "Results of a Two-Year Toxicity and Oncogenic Study of Chlorpyrifos Administered to CD-1 Mice in the Diet". Dow Chemical Toxicology Laboratory, 3/4/80. New information consists of individual data for blood smear exams, clinical observation and animal disposition, and gross and histopathology. Reviewer (Aldous) examined previously submitted chemical analyses of test material used in this and in one other study, and included evaluation in 5/29/87 review. No adverse effects noted. Study not acceptable, but possibly upgradeable. C. Aldous, 5/29/87.

342-013/053 031071 Summary only of 253:036340.

GENOTOXICITY

Bacterial reverse mutation assay ** (see after In vitro mammalian cell assay section for summary statement)

342-255 036348 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies," (brief summary) SRI, 1977; Salmonella and E. coli. UNACCEPTABLE with no adverse effect reported. Salmonella, 4 strains (no TA98), were tested with and without activation at 0, 1, 5, 10, 50, 100, 500 and 1000 µg/plate and with Escherichia coli at the same concentrations. Chlorpyrifos, 98.8%. No evidence of a cytotoxic concentration or rationale for maximum concentration used. No repeat trial, no individual plate counts if more than one was made. Not upgradeable. J. Gee, 2/13/86.

342-273 042784 Bruce, R. J. and J. A. Zempel, "Chlorpyrifos: Evaluation in the Ames' Salmonella/Mammalian-Microsome Mutagenicity Assay," Dow Chemical, Freeport, Texas, 1986; Salmonella. Chlorpyrifos (95.7%) tested in strains TA1535, TA1537, TA98 and TA100 at 0, 1, 3.16, 10, 31.6 and 100 µg/plate; with and without rat liver activation; 30 min preincubation before plating, triplicate plates, one trial, no evidence for increased reversion rate. UNACCEPTABLE. Report states that a precipitate formed at 100 µg/plate. The earlier study did not mention this. J. Gee, 7/30/86.

342-419 116728. Supplement to 042784. Contains individual plate counts and a revised table of contents. No change in the study status. No worksheet. Kellner and Gee, 7/9/93.

Mutagenicity: In vitro mammalian cell assay **

**342-255 036351 Mendrala, A. L., "Evaluation of Chlorpyrifos in the Chinese Hamster Ovary Cell-Hypoxanthine (Guanine) Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay," Dow Chemical, Midland, MI, Sept. 3, 1985. Chlorpyrifos, 95.7% purity, was tested at 0, 10, 20, 25, 30, 40 or 50 µM with and without activation for 4 hours. Positive control was 3 mM EMS. There were 5 dishes per treatment, in a single trial. A precipitate formed at 30 µM and above. Survival percentages (relative to 0 µM control) at chlorpyrifos levels of 10, 20, 25, 30, 40 or 50 were 92, 31, 23, 16, 9, and 7%, respectively. Testing thus bracketed practical limits based on both solubility and cytotoxicity. There was no increase in mutation frequency reported for chlorpyrifos in any single trial. Positive control mutation frequency was about 100x above background. Initially, results were considered to be negative for chlorpyrifos mutagenicity, however study was designated as unacceptable, based on lack of a confirming trial (see original review by J. Gee, 2/13/86). Current guidelines (OPPTS 870.5300, page 7) do not routinely require a repeat this assay after a negative response. Consistent with contemporary guidelines, study should be re-classified as acceptable, with no adverse effects. Aldous, June 5, 2015.

342-291 [No Record No., second "Mutagenicity" tab in volume]. Rebuttal comments ref 255:036351. CDFA conclusion was study still UNACCEPTABLE: major concern remaining is lack of a confirmatory test for a negative result. (J. Gee, 6/5/87).

342-291 057655 A table entitled "Analytical determination of stability of Chlorpyrifos in DMSO" in support of 255:036351, above. (Submitted as part of rebuttal document of 12/1/86).

***SUMMARY: The 1977 SRI study (#036348), using four strains of Salmonella (but not TA98) at 0 to 1000 µg/plate, was negative for increased reversion. Also, the CHO/HGPRT study on file showed negative results. EPA accepted this CHO study (#036351) although CDFA review found it unacceptable because there was no repeat. Considering all of these studies, with

no one alone being acceptable, and that #042784 is a repeat of #036348 -- the deficiency for which each was rejected separately -- the 842 data gap is considered filled.

Mutagenicity: In vivo cytogenetics **

342-419 116722 "Evaluation of Chlorpyrifos in an In Vitro Chromosomal Aberration Assay Utilizing Rat Lymphocytes", (Linscombe, V., Mensik D. and Clem, B., Dow Chemical Company, Lab Project Study ID: K-044793-092, 1/29/92). Chlorpyrifos, purity of 98.6%, was evaluated for clastogenic potential using rat lymphocytes treated for 4 hours with concentrations of 0 (DMSO), 5, 16.7, 50, 167.7, 500, 1667.0 or 5000 mg/ml (Assay 1) and 0, 5.0, 16.7, 50.0 and 167.0 mg/ml (Assay 2) with and without S-9 metabolic activation. Cultures were harvested 24 hours after treatment in Assay 1 and 24 and 48 hours after treatment in Assay 2. **No Adverse Effects: No increase in chromosomal aberrations at the highest scorable dose levels of 167 mg/ml (without S-9) and 50 mg/ml (with S-9). ACCEPTABLE. (Kishiyama, Kellner and Gee, 7/1/93).

342-739 161321 Exact duplicate of 342-419 116722 (above). This was submitted in a volume which contained primarily product chemistry data. Aldous, 11/12/98.

342-363 087919 McClintock, M. L., and B. B. Gollapudi, "Evaluation of Chlorpyrifos in the Bone Marrow Micronucleus Test." (Dow, TXT: K-044793-067A, 9/22/89). Chlorpyrifos, lot AGR 214637, 97.9%; tested with CD-1 (ICR) BR mice, with sacrifices of 5/sex/group at 24, 48 or 72 hours after a single oral gavage dosing of 0 (corn oil) or 90 mg/kg b. wt. stated to be 80% of the LD_{50}; cyclophosphamide as positive control; no mortalities but decrease in body weights in the treatment groups; no evidence of micronuclei formation and no clear effect on PCE/NCE. UNACCEPTABLE (only one dose level). (Gee, 3/12/90)

342-255 036350 Gollapudi, B. B., V. A. Linscombe, and J. E. Wilkerson, "Evaluation of Chlorpyrifos in the Mouse Bone Marrow Micronucleus Test," Dow Chemical, Freeport, Texas, 1985; Mouse micronucleus test. UNACCEPTABLE with no adverse effect. Chlorpyrifos, 95.7%, was given by oral gavage to 5/sex/group at 0, 7, 22, or 70 mg/kg with sacrifices at 24 and 48 hours. No statistically significant increase in micronuclei in PCE's is reported; % PCE marginally effected in females only at 48 hours being 63 as compared with 76 for the vehicle control. This is suggestive that a higher dose and/or a longer sampling time should have been included even at the risk of losing some of the animals. In the Appendix, data show that survival at 100 mg/kg would be adequate for the assay. Also, no clinical signs were observed. The high dose reportedly was based on 60% of the LD50 of approximately 111 mg/kg. Guidelines and the meaningfulness of the test call for some signs than a toxic dose was reached, either the MTD for the animal or cytotoxicity to the bone marrow. The only death was in female vehicle control. No data on micronucleated normochromatic erythrocytes are included. Because positive effects have been reported in gene conversion and DNA repair, an adequate test in this test area is needed. Not upgradeable. J. Gee, 2/13/86.

NOTE: EPA considers this study as acceptable, according to the EPA response to CDFA data gap status issues on chlorpyrifos, dated 1/17/89. Aldous, 12/4/89.

342-291 [No Record number, first "Mutagenicity" tab in volume]. Rebuttal comments ref 255:036350. CDFA conclusion was study still UNACCEPTABLE: major concerns remaining are inadequate justification of treatment levels, and lack of a 72 hr sacrifice time. J. Gee, 6/5/87.

Mutagenicity: DNA Damage (not a normally required test category) ** †

342-255 036349 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies," [Segment on mammalian *in vitro* unscheduled DNA synthesis assays] SRI, 1977; UDS in WI-38. UNACCEPTABLE but upgradeable with no adverse effect reported. Chlorpyrifos, 98.8%. WI-38, human embryonic lung fibroblasts, were exposed with and without activation (rat liver) to 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} with six cultures -S9 and 3 +S9. DPM/µg DNA is reported with no change in the DPM with increasing concentrations. DNA was extracted from the cells by a standard method and an aliquot used to determine the amount of DNA and another portion used to determine the incorporation of tritiated thymidine by liquid scintillation counting as a measure of DNA repair in response to damage by the test article. Missing information on how the CPM were converted to DPM, the quantity of DNA recovered per culture, the passage number of the WI-38, and the rationale for the selection of the concentrations used - whether solubility or cytotoxicity. CDFA review 2-13-86 J. Gee.

342-255 036347 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies --Microbiological Assays" (summary report), SRI, 1977; Saccharomyces cerevisiae D_3. UNACCEPTABLE with a positive effect reported. Mitotic recombination-gene conversion in yeast exposed to a 5% concentration for 4 hours, with and without metabolic activation. The test was repeated. No individual data. Because of the lack of data, the significance of the effect cannot be evaluated but the possible genotoxic effect must be noted. Upgradeable. J. Gee, 2/13/86.

342-255 042609 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies -Microbiological Assays" (summary), SRI, 1977; Escherichia coli and Bacillus subtilis [found under Tab 12, pg. 20]. UNACCEPTABLE with a positive adverse effect reported. Chlorpyrifos, 98.8% purity, at 2.5 µg/disc, was tested with E. coli W3110 and p3478 and with B. subtilis H17 and M45. No activation was included and the test reportedly was repeated 3 times. The comparable zones of inhibition between the strains indicated a larger zone for the repair defective strains. Only one value for each strain is reported. If the full report were submitted, it is possible that the effect could be evaluated for significance. Since no activation was included, the study is not upgradeable. J. Gee, 2/13/86.

**342-273 042785 Mendrala, A. L. and M. D. Dryzga, "Evaluation of Chlorpyrifos in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay," Dow Chemical, Midland, MI, 1986; Chlorpyrifos (95.7%); primary rat hepatocytes tested for unscheduled DNA synthesis at 10^{-6}, 3.13 x10^{-6}, x 10^{-5}, 3.16 x 10^{-5} and 1 x 10^{-4} M; triplicate cultures in a single trial; no evidence of UDS; toxicity at the highest concentration. Acceptable. J. Gee, 7/30/86.

SUMMARY: The positive findings in the two microbial studies are somewhat related. The B. subtilis test compares the response of rec^- (recombination defective) with wild type organisms.

The rec$^-$ strain is not as competent to repair damage and hence shows a greater inhibition of growth from lethality due to DNA damage. The test in Saccharomyces also measures recombination-type events in competent organisms and the increase in these events confirms the DNA damage. The complete versions of these two reports are needed to assess their significance. The two tests in mammalian cells measure a different repair event (excision repair) with repair replication occurring to fill the DNA gap following removal of damaged bases by excision using different enzymes. The positive findings in the microbial tests cannot be dismissed without more information about the bacterial studies.

REPRODUCTIVE TOXICITY, RAT **

**342-399 097570 "Chlorpyrifos: Two-generation dietary reproduction study in Sprague-Dawley rats", (W. J. Breslin, A. B. Liberacki, D. A. Dittenber, K. A. Brzak, and J. F. Quast). The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI., Study ID: K-044793-088, 6/5/91). Chlorpyrifos, (technical grade Dursban F insecticide, AGR 273801), 98.5% purity, was fed in the diet to 30 Sprague-Dawley rats/sex/group through 2 generations with 1 litter per generation. Concentrations were adjusted as needed to achieve exposures of 0, 0.1, 1.0, and 5.0 mg/kg/d. Treatment began approximately 10 and 12 weeks prior to breeding for the F0 and F1 adults, respectively. Cholinesterase (ChE) inhibition NOEL = 0.1 mg/kg/d (Plasma and RBC AChE inhibition at 1.0 and 5.0 mg/kg/d). Parental NOEL = 1.0 mg/kg/d (increased degree of vacuolation in zona fasciculata, especially in males; altered tinctorial properties in this tissue in females). Reproductive NOEL = 1.0 mg/kg/d (slightly reduced pup weights and slightly reduced pup survival at 5.0 mg/kg/d). There were no clinical signs specifically indicating ChE inhibition. The reproductive findings at 5 mg/kg/d do not warrant a "possible adverse effects" designation, since brain ChE levels were very markedly depressed at that dose level, and all observed reproductive effects appeared to be due to failure of dams to nurture pups which were otherwise normal. ACCEPTABLE. (Green and Aldous, 5/11/92).

342-685 152365 Exact duplicate of 342-399 097570.

342-374 090493 Interim report for Record No. 097570, above.

342-686 152368 Breslin, W. J., A. B. Liberacki, D. A. Dittenber, and J. F. Quast. "Evaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat". *Fundam. Appl. Toxicol*. **29**:119-130 (1996). This is a published summary of major findings of two accepted studies: the reproduction study above (342-399 097570) and the rat teratology study (342-254 036344). Since the abstract was consistent with DPR 1-liner conclusions for the two studies, this publication was not independently reviewed. Aldous, 7/31/97.

342-254 036341 "Three Generation Reproduction and Teratology Study in the Rat Following Prolonged Dietary Exposure to Dursban, O,O-Diethyl O-3,5,6-Trichloro-2-Pyridyl Phosphorothioate," Dow Chemical, Zionsville, Indiana, 8/20/71. Chlorpyrifos, purity and grade not specified. Doses for the main portion of the reproduction study were 0, 0.1, 0.3, and 1.0 mg/kg/d in diet. ChE inhibition NOEL= 0.3 mg/kg/d. General adult toxicity NOEL = 1.0 mg/kg/d (HDT). Reproductive NOEL = 0.3 mg/kg/d (slightly increased pup mortality in first 5

days post-partum) UNACCEPTABLE, incomplete, not upgradeable (more definitive follow-up study is 254:036343). C. Aldous, 1/31/86.

(An additional copy of 036341 is found in Document No. 342-685, Tab 49 (no record #).

EPA 1-liner: [3-Generation reproduction/teratology - rat; Dow Chem. Co.; 8/20/71] Reproduction NOEL>1.0 mg/kg/d (HDT); Teratogenic NOEL = inconclusive. ChE NOEL=0.1 mg/kg Core grade, minimum

342-254 036343 Dietz, F. K., D. C. Mensik, C. A. Hinze, B. L., Rachunek, and H. W. Taylor, "Dursban Insecticide: Assessment of Neonatal Survival In A Two-Generation Reproduction Study In Rats," Dow Chemical, Freeport, Texas, 7/83. Chlorpyrifos, technical; 0, 0.5, 0.8, and 1.2 mg/kg/d (dietary). Parental toxicity NOEL = reproductive toxicity NOEL = highest dose tested = 1.2 mg/kg/d. UNACCEPTABLE, incomplete, upgradeability unlikely (highest dose level not demonstrably toxic, and no justification offered for dosage selection). C. Aldous 2/7/86.

EPA 1-liner: [Two generation repro - rat; Dow Chem.: 7/83] Reproductive NOEL > 1.2 mg/kg/d (HDT); Systemic NOEL = 0.8 mg/kg; Systemic LEL= 1.2 mg/kg (decreased weight gain); Core grade, supplementary.

342-681 152366 Exact duplicate of 254 036343, above.

342-291: [No Record #, Tab = "Reproduction"] Rebuttal comments ref. rat reproduction studies 254:036341 and 254:036343. Registrant noted that CDFA should consider both reproduction studies together, considering additionally rat chronic data. Registrant suggested that plasma and RBC AChE inhibition data support adequacy of dose. CDFA response: Doses are not justified in terms of parental toxicity, notwithstanding enzyme inhibition effects. Chronic studies are imperfect surrogate studies for evaluation of microscopic changes due to test article, since in chronic studies there is no evaluation of effects which carry over the generations. No change in status of studies. C. Aldous, 6/2/87.

342-686 152367 James, P., A. Stubbs, C. A. Parker, J. M. Offer, A. Anderson, "The effect of Pyrinex (chlorpyrifos) on reproductive function of two generations in the rat", Huntingdon Research Centre, Ltd., 4/22/88. HRC Report # MBS 29/881452. Crl:CD®(SD)BR rats received diets containing 0, 2, 10, or 50 ppm chlorpyrifos (95% purity) in diets over 2 generations (1 litter per generation). Parental rats numbered 28/sex/group in the F0 generation, and 24/sex/group in the F1 generation. Protocol was that of a standard reproduction study, with a few pre-weaning developmental evaluations added (surface righting, air righting, and startle responses; and pupil reflex). There were **no definitive treatment-related effects** (report attributes 3 high dose deaths to treatment, however there were deaths in other groups and no evident unique symptoms in high dose decedents). Study is **not acceptable** as presented (report evidently contains 401 pages, but only pp. 1-228 are present, "confidentiality" stamps cover much of the text, more definitive high dose justification would be needed, and histopathology of parental rats is needed if this study is to be upgraded). Aldous, 8/22/97.

DEVELOPMENTAL TOXICITY

Rat Developmental Toxicity **

**342-254 036344 Ouellette, J. H., D. A. Dittenber, P. M. Kloes, and J. A. John, "Chlorpyrifos: Oral Teratology Study in Fischer 344 Rats," Toxicology Research Lab., Dow Chemical USA, Midland, MI, 7/5/83. Chlorpyrifos, 96.6%. 0, 0.1, 3.0, and 15 mg/kg/d (gavage). Maternal NOEL (excluding cholinesterase (ChE) inhibition) = 3.0 mg/kg/d (cholinergic effects). Maternal ChE inhibition NOEL = 0.1 mg/kg/d (inhibition of plasma and RBC AChE). Developmental toxicity NOEL = 15 mg/kg/d (HDT). ACCEPTABLE due to submission of supplementary information. See CDFA Rebuttal comments, C. Aldous, 6/1/87. (Study had been classified unacceptable in previous review by C. Aldous 2-10-86). C. Aldous, 6/1/87.

EPA 1-liner: [Teratology - rat; Toxicology. Research Lab; 7/5/83] Teratogenic and fetotoxic NOEL> 15 mg/kg/d (HDT); Maternal NOEL= 0.1 mg/kg; Maternal LEL= 3.0 (ChE inhibition) Core grade, minimum.

342-683 152360 (exact duplicate of 342-254 036344, above).

342-291 050624 (Rebuttal by Ouellette *et al*. to primary study 254:036344). Considered in 6/1/87 review of primary study, 254:036344, above.

342-291 050625 (Pilot study to primary study 254:036344). Ouellette, J. H., D. A. Dittenber, R. J. Kociba, and J. A. John, "Chlorpyrifos: Oral teratology probe study in rats". Toxicology Research Lab, Dow, 1/4/83.

Chlorpyrifos, 96.6%. 0, 3, 10, and 30 mg/kg/d by gavage in cottonseed oil. Study demonstrates that 30 mg/kg/d is severely toxic to dams: maternal deaths, typical cholinergic signs, high number of resorptions. Slightly matted haircoat and slight enlargement of adrenals were observed at 15 mg/kg/d. This pilot study clearly substantiates the adequacy of the dosage range selected for the primary study, 254:036344. C. Aldous, 6/1/87.

342-695 153117 Rubin, Y., N. Gal, T. Waner, and A. Nyska, "Pyrinex teratogenicity study in the rat", Makhteshim-Agan of North America Inc., 7/15/87. Laboratory Study #MAK/101/PYR. At least 21 pregnant CD rats/group were dosed with Pyrinex Technical (chlorpyrifos), purity 96.1% by gavage in corn oil on days 6-15 p.c. at 0, 0.5, 2.5, or 15 mg/kg/d. No maternal ChE NOEL was identified (dose-related plasma ChE inhibition at all dose levels at day 15 p.c., with restoration of normal ChE activity in all but high dose dams by p.c. day 20. Maternal functional NOEL = 2.5 mg/kg/d (tremors in 3/21 dams, transient food consumption reduction, modest but consistent body weight decrement). Developmental NOEL = 2.5 mg/kg/d (slight increase in early resorptions). **No adverse reproductive effect at dose levels sufficient to elicit cholinergic responses. Acceptable. Aldous; May 1, 1997.

342-683 152361 Exact duplicate of 342-695 153117, above.

342-681 152354 Muto, M. A., F. Lobelle, J. H. Bidanset, and J. N. D. Wurpel, "Embryotoxicity and neurotoxicity in rats associated with prenatal exposure to Dursban", *Veterinary and Human Toxicology* 34, 498-501 (1992). Investigators from the Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY. Test article was a formulation of 1% chlorpyrifos, 6%

xylene, and 93% water. Suspensions were diluted to an unspecified dosing volume with saline. Dosing was ip, either on days 0-7 or on days 7-21 at dose levels of 0, 0.03, 0.1, or 0.3 mg/kg/d of chlorpyrifos. In most cases, there were 8 pregnant rats (strain unspecified) per dose for each treatment time period. Dams were allowed to litter, then pups were evaluated for "general viability, body weight and physical characteristics". Selected pups were evaluated for "neurotoxicity" on a rotorod on day 16. The same day, pups were evaluated for motor behavior (subjective open field observation) and for righting behavior on an inclined screen. An additional study evaluated the neurotoxicity and behavioral tests following exposures of 0.1 or 0.3 mg (presumably ip) as single doses on day 3, 10, or 12 postpartum, or as multiple doses on days 6-10 postpartum. Investigators claimed that treatment caused increased embryolethality following dosing on gestation days 0-7 and gestation days 7-21. Since the highest embryolethality was in the lowest dose group treated on gestation days 0-7 (77% lethality), these data are of questionable value. Incidences of "physical abnormalities" were reportedly highest in 0.1 and 0.3 mg/kg/d groups (66 and 55%, respectively), among litters treated on gestation days 0-7. No corresponding control data were presented. Rotorod performance was reported to be impaired in pups dosed at 0.3 mg/kg on days 3, 10, and 12, and in offspring of dams dosed with 0.3 mg/kg on days 7-21, and in offspring of dams dosed with 0.03, 0.1, or 0.3 mg/kg on days 0-7. These data are suspect because differences between mean values at any treatment time dwarfed differences between dose groups at individual treatment times, even though all pups were evaluated at day 16. The study is unacceptable (in addition to deficiencies noted above, test article does not represent either the AI or any end use product; the route (ip) is not a plausible route of human exposure; the conclusions are speculative, evidenced by discussion of possible delayed distal neuropathy, while ignoring a valid 1986 subchronic hen neurotoxicity study, which would have been available through "freedom of information" provisions long before the time of this publication; and the presentation of the article shows that it could not have gone through a meaningful review, indicated by the above deficiencies, and by misspellings (the term "access" when "assess" was meant) and by failures to provide control data in figures or to provide numerical counts for types of purported treatment-caused malformations. No more information is requested of this paper. Aldous, 9/3/97.

342-681 152355 Nimphius, M. J. (M.S. dissertation under direction of graduate advisor J. H. Bidanset at St. John's University College of Pharmacy and Allied Health Professions, New York). "The effects of chlorpyrifos and xylene on embryonal and fetal development in the rat" (approval date: 9/13/95). Sprague-Dawley rats were dosed subcutaneously with 0, 0.3, 3, or 10 mg/kg/d chlorpyrifos (analytical grade, 99% purity) on days 1-7 of gestation (typically 8/dose/group), then sacrificed on gestation day 19 or 20. Other rats received xylene or chlorpyrifos/xylene s.c. on the same schedule. Parameters examined were resorptions, weights and lengths of fetuses, and external malformations. None of these showed biologically meaningful changes. This study is unacceptable (it does not conform to any FIFRA study design: route is not relevant to plausible human exposure, timing of dosing is not useful for evaluation of malformations, fetal examinations were only for grossly evident changes, group sizes were too small, and sacrifices were not done on a fixed gestation day). The study does not make a significant contribution to chlorpyrifos hazard assessment. Aldous, 9/3/97.

[Rat Developmental Toxicity Studies: Chlorpyrifos Metabolites]

342-684 152362 Hanley, T. R., G. J. Zielke, and L. G. Lomax, "3,5,6-Trichloro-2-pyridinol: oral teratology study in Fischer 344 rats", The Dow Chemical Co., Midland, MI, 7/23/87. Laboratory Study #: K-038278-011. Groups of 32-34 mated Fischer 344 rats were dosed with 0, 50, 100, or 150 mg/kg/d 3,5,6-trichloro-2-pyridinol (TCPy, 99.7% purity) by gavage in 4 ml/kg Methocel on days 6-15 of gestation in a standard teratology study. Maternal NOEL = 50 mg/kg/d (minor body weight gain decrements). Developmental NOEL = 150 mg/kg/d (HDT). An acceptable study of a major metabolite of chlorpyrifos, with no adverse effect indicated. Aldous, 7/31/97.

Rabbit Developmental Toxicity ** (No adverse effects for technical chlorpyrifos, however high doses of a metabolite caused developmental toxicity)

342-694 153116 Rubin, Y., A. Nyska, and T. Waner, "Pyrinex teratogenicity study in the rabbit", Life Science Research Israel Ltd., 7/15/87. Laboratory Study # MAK/103/PYR. At least 14 HY/CR (a NZW variety) rabbits per group were dosed by gavage in corn oil with chlorpyrifos (Pyrinex Technical, purity 96.1%) on days 7-19 p.c. at 0, 1, 9, 81, or 140 mg/kg/d. Maternal NOEL = 81 mg/kg/d (body weight gain decrement during treatment period). Developmental NOEL = 81 mg/kg/d [reduced crown/rump length, reduced fetal weight, ossification delays (indicated by non-ossification of fifth sternebra and/or xiphisternum)]. No adverse effects are indicated. For comparison, the pilot study had found 100% lethality in does at 270 mg/kg/d. **Acceptable. Aldous, 4/29/97.

342-685 152364 Exact duplicate of 342-694 153116, above.

[Rabbit Developmental Toxicity Studies: Chlorpyrifos Metabolites]

342-684 152363 Hanley, T. R., G. J. Zielke, and L. G. Lomax, "3,5,6-Trichloro-2-pyridinol: oral teratology study in New Zealand White rabbits", The Dow Chemical Co., Midland, MI, 7/23/87. Laboratory Study #: K-038278-015. Sixteen does/group were dosed with 0, 25, 100, or 250 mg/kg/d 3,5,6-trichloro-2-pyridinol (TCP, purity 99.7%) by gavage in aqueous 0.5% Methocel on gestation days 7-19 in a teratology study. Maternal NOEL = 100 mg/kg/d (minor maternal body weight decrement during treatment). Developmental NOEL = 25 mg/kg/d (hydrocephaly and dilated cerebral ventricles). The latter observations were not statistically significantly increased in either of the two higher dose groups compared to concurrent controls, however historical background incidences were very low (compare hydrocephaly litter incidences of 2/13 and 3/13 at 100 and 250 mg/kg/d, respectively, to a historical incidence of 1/839 litters). These findings indicate a **possible adverse effect**. For perspective, 100 mg/kg/d of TCP is the molar equivalent to 66% of a chlorpyrifos dose which caused 100% mortality in LSRI Report MAK/102/PYR (cited in the accepted chlorpyrifos rabbit teratology study under DPR Record No. 153166). **Acceptable** metabolite study. Aldous, 7/31/97.

Mouse Developmental Toxicity **

**342-254 036345 Deacon, M. M., J. S. Murray, M. K. Pilny, D. A. Dittenber, T. R. Hanley, Jr., and J. A. John, "The Effects of Orally Administered Chlorpyrifos on Embryonal and Fetal Development in Mice," Dow Chemical, Toxicology Research Lab., Midland, MI, 7/24/79; Chlorpyrifos, presumed technical; 0, 0.1, 1, 10, and 25 mg/kg/d by gavage; NOEL for maternal

functional toxicity = 1 mg/kg/d [cholinesterase (ChE) effects as salivation, tremors, etc.]. ChE enzyme NOEL = 0.1 mg/kg/d (significant inhibition of maternal plasma ChE at 1 mg/kg/d). Developmental toxicity NOEL = 10 mg/kg/d (decreased fetal length and weight, delayed ossification in skull, sternebrae). ACCEPTABLE, in consideration of additional information in 291:050626 (See one-liner below). Report was previously not accepted (CDFA review 2/13/86, C. Aldous). C. Aldous, 6/1/87.

342-291 050626 (Addendum to 254:036345, primary mouse teratology study). Dow Chemical, Midland, MI, 7/24/79. New information provides grade of test article, dates of preparation of dose solutions, individual necropsy sheets for dams dying prior to term, and rationale for selection of mouse as test animal. C. Aldous, 6/1/87.

EPA 1-liner: Teratology - mice; Toxicology. Research Lab.; 7/24/74 [sic: presumed this is the 7/24/79 study]; Teratogenic NOEL > 25 mg/kg/d (HDT); fetotoxic NOEL = 10 mg/kg fetotoxic LEL = 25 mg/kg (decreased fetal length, increased skeletal variants); Plasma and RBC AChE NOEL = 0.1 mg/kg/d.

342-013/053 031072 Summary of 254:036345 (see above).

342-682 152359 (Tab 43). Deacon, M. M., J. S. Murray, M. K. Pilny, K. S. Rao, D. A. Dittenber, T. R. Hanley, Jr., and J. A. John, "Embryotoxicity and Fetotoxicity of Orally Administered Chlorpyrifos in Mice", *Toxicol. Appl. Pharmacol.* 54:31-40 (1980). This is the published report corresponding to 342-254 036345, above.

Developmental Toxicity: Allegations of Effects on Humans

The following critical review by Dr. J. E. Gibson and associated support documents were submitted in response to allegations that chlorpyrifos elicited human malformations

342-680 152356 Gibson, J. E., "Critical review of allegations associating Dursban with human teratogenicity", 12/23/96 (analysis was given DowElanco Study ID JEG122396). Dr. Gibson was responding to allegations by Dr. J. Sherman that chlorpyrifos was the causative agent for several human birth defects. The most detailed version of Dr. Sherman's report was in Int. J. Occup. Med. Toxicol., 4:417-431 (1995). Dr. Gibson's primary objections to the article were (1) Dr. Sherman does not have the training and experience to properly perform such an analysis, (2) the four cases described do not present a coherent pattern of effects, (3) the possibilities of genetic causation were ignored, even though in most cases one or more physicians experienced in evaluation of birth defects attributed findings to genetic defects (4) none of the cases offered measures of exposure, (5) statistical analysis in the article was unsound, (6) outcomes of cited animal studies were misunderstood or misrepresented, and (7) the article did not state the author's role as paid consultant in lawsuits filed by the three affected families, which disclosure is an ethical responsibility of authorship. All lawsuits involving the four children have been dismissed. Neither the Sherman report (DPR Record No. 152349) nor Dr. Gibson's review are primary sources of new data, hence do not have independent worksheets. **Supporting data, including some complete studies, follow in Document Nos. 342-681 to 342-686. "One-liners" describing these submissions are found in this worksheet.** Aldous, 8/22/97.

Records submitted in support of 342-680 152356 above, included: Document No. 342-681: Record Nos. 152349, 152350, 152351, 152352, 152353 152354,152355; and Document No. 342-682: Record Nos. 152357, 152358, 152359.

NEUROTOXICITY

Acute neurotoxicity, rat **

342-448 126408 Wilmer, J., et. al. "Chlorpyrifos: Acute Neurotoxicity Study in Fischer 344 Rats", (Dow Chemical Company, Study ID: K-044793-093B, 9/11/92). Chlorpyrifos (purity 98.1%, lot #MM-890115-616) was administered in a single oral gavage to 10 Fischer 344 rats/sex/group at levels of 0, 10, 50 or 100 mg/kg. Body weights of mid- and high-dose rats were significantly reduced on day 2 but not on day 8 or 15. Clinical signs (increased perineal soiling) in mid- and high-dose rats and FOB observations (incoordination, decreased muscle tone, tremor, increased lacrimation and salivation) in high-dose females were seen soon after dosing (day 1). Motor activity was reduced in mid- and high dose rats on day 1; some reductions persisted to day 8 in high-dose females. NOEL (Body wt., Clinical signs, FOB and motor activity) = 10 mg/kg. No histopathologic changes. NOEL (histopathology) = 100 mg/kg. **No Adverse Effects. Original DPR review had requested additional purity, stability and homogeneity data on the dosing material, justification for dose level selection, and clarification of the statistical methods used, as criteria for "acceptable" status. These data were provided (see review for Record No. 132457, below) and report is now **acceptable**. This study type is classified as "supplemental" for SB 950 at this time. Kellner and Gee, 7/5/94; Aldous, 4/9/97.

342-492 132457 [Cover letter referencing supplementary data was by Blewett, T. C. The acute range-finding study in this record supporting dose selection for the acute neurotoxicity study was by Wilmer, J. W. *et al.* (Study ID K-044793-093A)]. Addendum to Document # 342-448, Record # 126408 (rat acute neurotoxicity). Cover letter date: 10/4/94. The three primary acceptability concerns expressed in the original DPR review have been adequately addressed: characterization of technical and treated diets for content, stability, and homogeneity; range finding study clinical signs data as evidence that selected dose levels were appropriate; and evaluation of statistical significance for major parameters of this study. In the range-finding study, two F344 rats/sex/group were dosed once by corn oil gavage at 50, 100, 150, and 200 mg/kg. Clinical signs consistent with ChE inhibition peaked at about 6 hr after dosing. Major signs were decreased activity, incoordination, lacrimation, muscle twitches, perineal soiling, salivation, and tremors. These signs were well established at 100 mg/kg and above, especially in females. Range finding study data are sufficient to justify dose levels used in the neurotoxicity study. Additional statistical data are consistent with interpretations in the original DPR review. The study is re-classified as **acceptable**, with **no adverse effects** other than expected ChE inhibition-associated changes. Aldous, 4/9/97.

90-day neurotoxicity, rat **

**342-445 126304, "Chlorpyrifos: 13-Week Neurotoxicity Study in Fischer Rats", (Shankar, M., Bond, D. and Crissman, J., Dow Chemical Company, Laboratory Project K-044793-094, 9/16/93). Chlorpyrifos, purity 98.1%, was administered in the feed at concentrations of 0, 0.1, 1, 5 or 15 mg/kg to 10 Fischer 344 rats/sex/group for 13 weeks. High-dose males and females had

reduced motor activity at week 4. Perineal soiling (low incidence) was observed for 5 and 15 mg/kg/d groups; NOEL (for clinical signs, FOB, motor activity) = 1 mg/kg/d. No histopathologic findings. Neuropathological NOEL = 15 mg/kg/d. **No Adverse Effects.** Report was originally classified as unacceptable, but upgradeable. Data provided in Record No. 132458 (see below) allowed an upgrade to **acceptable** status. This study type is considered "supplemental" under SB 950 at this time. Kishiyama, Kellner and Gee, 7/6/94; Aldous, 4/8/97.

342-493 132458 (Addendum to Document # 342-445, Record # 126304). Cover letter dated 10/4/94. The three primary acceptability concerns expressed in the original DPR review have been adequately addressed: characterization of technical and treated diets for content, stability, and homogeneity; ChE inhibition data as evidence that selected dose levels were appropriate; and evaluation of statistical significance for major parameters of this study. Data obtained from a 1988 subchronic feeding study found ChE enzyme inhibition NOEL = 0.1 mg/kg/d (inhibition of plasma ChE in both sexes and of RBC AChE in females at 1 mg/kg/d). ChE-related clinical effects NOEL = 1 mg/kg/d (perineal staining in occasional females at 5 and 15 mg/kg/d). Motor activity reduction, at 15 mg/kg/d during the week 4 evaluation only, was confirmed statistically. NOEL for findings other than probable acute ChE effects = 15 mg/kg/d (HDT). The study is re-classified as **acceptable**, with **no adverse effects** other than expected ChE inhibition and associated changes. Aldous, 4/8/97.

342-448 126409 Spencer, P. *et. al*. "Positive Control Exercises: Motor Activity, Functional Observational Battery and Neuropathology". Dow Chemical Co. submitted this report in support of -445:126304 and -448:126408; it contains validation studies of motor activity tests, functional observational battery (FOB) assays and neuropathological examinations using rats that were administered compounds with well-documented neurotoxic potential. This document was found to be ACCEPTABLE to satisfy the FIFRA guidelines for positive controls. An evaluation of these studies is included in the background sections of the acute and 13-week rat neurotoxicity studies mentioned above. No Worksheet. Kellner and Gee, 7/18/94.

4-week rat oral gavage cognitive study **

**342-747 162522 Maurissen, J. P., M. R. Shankar, and J. L. Mattsson, "Chlorpyrifos: cognitive study in adult Long-Evans rats", The Dow Chemical Co., Midland, MI, 4/29/96, Laboratory Project ID: K-044793-096. Female Long-Evans rats were dosed by gavage in corn oil with 0, 1, 3, or 10 mg/kg/d chlorpyrifos (98.1% purity) for 4 weeks. The cognitive study was a "delayed matching to position task" design. Cognitive testing was done during each of the treatment weeks and for 4 weeks thereafter, by methods described below. Rats were placed on modest food restriction to provide incentive to seek the "food reward" in the study. Rats were trained and selected for the study, based on positional memory performance. In a given test, a rat was presented with one of two retractable levers. The rat was to press the lever offered, cross the cage and interrupt a beam at the food cup within 10 seconds, and then return to the side of the cage with the levers. At this time, both levers would be presented. The rat was expected to select and press the correct lever (i.e., the one just presented a few seconds earlier) within 10 seconds after leaving the food cup station. A correct choice made a food reward available at the food cup. In addition to the above test, the task was made more difficult by involving progressively longer delays (up to 15 seconds) between the first lever press and the time in which a nose-poke in the food cup would extend the levers (called the delayed matching-to-position or

"DMPT" paradigm). These rats were also examined twice daily on treatment days during the 4-wk dosing period: observations were about 3 hr and 21 hr after the most recent treatment. Satellite groups of 6/dose/interval were used for ChE assays and brain NTE assays on the day following the last treatment, and 1 month after the last treatment. The 1998 DPR review placed the NOEL for memory retention at 3 mg/kg/d (considering a small apparent memory retention change at 10 mg/kg/d to be a "possible adverse effect"). **This determination was subsequently changed** (see review for Document No. 342-789, immediately below). NOEL for clinical observations is 1 mg/kg/d (miosis). There is no NOEL for ChE inhibition (marked inhibition of plasma and RBC AChE and modest (8%) inhibition of brain ChE at 1 mg/kg/d). Some high dose observations associated with the DMPT tests were appropriately considered by investigators to have been attributable to motor slowing and/or decreased motivation (increased "actual total delay", increased "void trials", and decreased numbers of nose-pokes per trial). None of these were noted after the end of the treatment period. Report was originally classified as not acceptable (requiring dosing solution analysis). Such data were subsequently provided (see immediately below). Study is **acceptable**. Aldous, 11/6/98, 10/12/99.

342-789 168961, 168962, and 168963. **Supplemental information to the above cognitive study (Record 342-747 162522)**. Additional data and explanatory text were provided. Essential responses summarized below are detailed in review "W162522 s01.wpd". New data supplied dosing solution analyses, and additional tables showing mean correct responses for individual animals and for treatment groups, including methodology used to obtain memory retention slope values. **These data allow an upgrade of Record No. 162522 to acceptable status.** In addition, investigators provided a statistical analysis of slopes of the memory retention curves for the various treatment groups. Data show that there were no statistically significant responses, hence data **do not demonstrate a possible adverse effect** (a change from the previous review). The variability of the data is sufficiently large that only a very substantial decrease of memory retention would have been detectable, thus the present study conditions **did not provide a sensitive test**. Aldous, 10/12/99.

Developmental neurotoxicity, rat **

**342-746 162521, Hoberman, A. M., "Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®(SD)BR VAF/Plus® presumed pregnant rats", Argus Research Laboratories, Inc., 5/1/98. Sponsor Protocol No. K-044793-109; Argus Study ID 304-001. Crl:CD®(SD)BR VAF/Plus® presumed pregnant rats were gavaged on gestation day 6 through lactation day 11 with chlorpyrifos (99.8%) in corn oil at 0, 0.3, 1, and 5 mg/kg/d. Initially there were 25 dams/group on treatment. On lactation day 5, twenty litters/treatment were continued on study. Four subsets of 20 pups/sex/group were selected on lactation day 5, each consisting of 1/sex/litter. Primary investigations for the subsets were: (Subset 1): morphometric evaluations and histopathology of brains after postpartum day 12 sacrifice, (Subset 2): spatial delayed alternation studies at postpartum days 23-25 and 62-91, (Subset 3): motor activity testing on postpartum days 14, 18, 22, and 61: auditory startle on postpartum days 23 and 62, (Subset 4): evaluation of developmental landmarks (pinna unfolding, eye opening, preputial separation or vaginal opening); brain weight evaluation in 10/sex/group sacrificed during lactation days 66-71, and neurohistopathology following *in situ* perfusion of 6/sex/litter. Maternal NOEL = 0.3 mg/kg/d (brain ChE inhibition). Clinical signs of ChE inhibition were observed in 5 mg/kg/d dams. Developmental NOEL = 1 mg/kg/d (decreased neonatal survival;

decreased pup growth, with 11% reduction in body weight at 66 days postpartum in males; maturational delays of pinna unfolding, preputial separation in males, and vaginal patency in females; reduced morphometric dimensions of cerebellum and hippocampal gyrus at day 12 postpartum compared to concurrent and historical controls, reduced morphometric dimensions of parietal cortex and hippocampal gyrus at day 66 postpartum compared to concurrent and historical controls in high dose females, reduced motor activity at day 14 postpartum, reduced auditory startle habituation peak response and increased latency to response at day 23 postpartum). This study was classified as "not acceptable but upgradeable" in the initial review, with the primary concern being appropriateness of the validation studies for evaluation of spatial delayed alternation. The response in Record No. 168955 (below) addressed the advantages of the using memory retention as a function of time for validation of technique, as compared with memory reduction due to exogenous chemicals. The investigators' response gave examples of many confounding effects of exogenous chemicals on parameters other than on memory. Study findings are not of sufficient magnitude or persistence to be considered as "adverse". Report is now **acceptable**. Aldous, 11/13/98 and 9/17/99.

342-769 164347 Submission of morphometry and histopathology data on F1 rats sacrificed after day 66 in Record No. 162521, above. Data were incorporated into the review for the main study under that Record Number. Aldous, 11/12/98.

342-789 168955, 168959, and 168960. Supplemental information to developmental neurotoxicity study 342-746 162521. Final report date of update: 5/7/99. Additional data and explanatory text were provided, **allowing an upgrade of Record No. 162521 to acceptable status**. Essential responses summarized below are detailed in review "s162521 s01.wpd". The validation studies for evaluation of spatial delayed alternation, which were based on temporal patterns of memory performance over sufficient duration to show a consistent linear change over time, were shown to be satisfactory. Representative micrographs prepared by the pathologist were presented, demonstrating several of the commonly encountered lesions following insult to the several areas of the CNS, dorsal root ganglia, and peripheral nerves. Additional brain morphometric data requested by US EPA were provided, plus selected published articles. One article showed that poor nutrition reduces pup brain weight increases, although to a much lesser extent than the decrement of body weight gain. Another article determined that the reductions of dimensions in brain regions appear to affect all brain morphometric measurements proportionately. A third article showed that poor nutrition leads to locomotion delays which are quite remarkable during lactation days 14-16, whereas some components of coordinated movement and altered posture remain affected for a longer time. Aldous, 9/17/99.

342-832 (suppl. to 342-746) 182481 (suppl. to 162521) Hoberman, A. M., Report Supplement 3 to: "Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®(SD)BR VAF/Plus® presumed pregnant rats, "Argus Research Laboratories, Inc., dated 5/1/98 (of original study), this supplement dated Oct. 9, 2000. Protocol No. of this supplement: 304-001. Brain morphometric data from the original report were re-tabulated alongside historical control data from 4 or 5 studies per parameter. Only one measurement having a high dose value statistically significantly different from concurrent controls was outside the range of the historical controls: the cerebellar anterior/posterior dimension in 5 mg/kg/d male 12-day pups was significantly below concurrent control dimension, and also outside the range of the available historical controls. Females did not suggest such a relationship at 12 days, and neither sex

showed altered cerebellar anterior/posterior distance after 66 days. In the context of the demonstrated high maternal and neonatal toxicity of this dose, the supplemental data reinforce the lack of demonstrated special toxicity of the test article toward the developing nervous system. Supplemental to a previously acceptable study with no adverse effects. Aldous, 9/26/01.

342-824 178362 [Same report as 342-746 162521, above].

Delayed neurotoxicity, hen **

**342-291 051119 Barna-Lloyd, T., J. R. Szabo, and J. T. Young, "Chlorpyrifos: Subchronic Organophosphate-Induced Delayed-Neurotoxicity (OPIDN) Study In Laying Chicken Hens," (Report No. TXT:K-044793-064), Health & Environmental Sciences, Dow Chemical, Freeport, Texas, 4/86. Chlorpyrifos, tech. (approx. 96% purity). 0, 1, 5, and 10 mg/kg/d. No evidence of delayed distal neuropathy. 10 mg/kg/d chlorpyrifos caused weight loss, diminished egg laying capacity, and transient abnormal gait (fully reversible between dosing periods, and not persistent throughout study). Study fills neurotoxicity data requirement. C. Aldous, 6/3/87.

342-255 036346 Rowe, L. D., S. D. Warner, and R. V. Johnston, "Acute Delayed Neurotoxicologic Evaluation of Chlorpyrifos in White Leghorn Hens," Dow Chemical, Lake Jackson, Texas, 5/22/78; Chlorpyrifos, tech; 0, 50, and 100 mg/kg (gelatin capsule); NOEL = 100 mg/kg for behavioral or microscopically evident delayed neuropathy (Highest dose tested) NOT ACCEPTABLE, not complete, not upgradeable (no repeat dosage at day 21 when no effects were observed, not all currently required tissues examined.) C. Aldous, 2/13/86.

EPA 1-liner: [Acute delayed neurotoxicity - hen; Dow; 5/22/78] LD50 in hens= 50 mg/kg Negative @ 50 & 100 mg/kg. Core grade, minimum.

342-496 132855 Abou-Donia, M. B., and K. R. Wilmarth, "DowElanco chlorpyrifos joint neurotoxic action of chlorpyrifos and safrotin in hens (Duke Univ. Medical Center Dept. of Physiology and Pharmacology, Durham, NC). Assigned to Worker Health and Safety Branch for review. (Aldous, 8/8/97).

342-745 162520 (No Author) "Preliminary Report: Assessment of neurotoxicity associated with co-exposure to the organophosphorus insecticides chlorpyrifos and diazinon". White leghorn hens were dosed with maximal levels of chlorpyrifos and/or diazinon and kept alive with atropine and 2-PAM for 96 hours prior to sacrifice and assays of ChE (plasma and brain), and brain NTE. There were apparently cumulative effects for brain and plasma ChE. Although diazinon by itself did not affect NTE activity, diazinon potentiated the NTE inhibition of chlorpyrifos from 35% to 65% of normal. There is insufficient information in this preliminary report to warrant a Medical Toxicology Branch worksheet. Aldous, 11/09/98.

IMMUNOTOXICITY **

** 342-0907; 258212; AChlorpyrifos: Assessment of Immunotoxic Potential Using the Sheep Red Blood Cell Assay after 28-Day Dietary Exposure to Rats@; (D.R. Boverhof, J.A. Murray, R. Sura; Toxicology & Environmental Research and Consulting, The Dow Chemical Company,

Midland, MI; Study ID No. 101023; 6/28/10); Ten female Sprague-Dawley rats/group received 0, 0.4, 2.0 and 10.0 mg/kg/d of Chlorpyrifos technical (lot no. KC28161419; purity: 99.8%) in the diet for 28 days. Another 10 females were dosed by intraperitioneal injection with 20 mg/kg/d of cyclophosphamid from day 24 through day 28 as the positive control group. No deaths occurred during the treatment period. There was no treatment-related effect upon the mean body weights or food consumption. The hematology parameters were not affected by the treatment. Red blood cell cholinesterase (ChE) activity was reduced in a dose-related manner for all treatment groups. Brain ChE activity was significantly less than that of the controls at the 2 and 10 mg/kg treatment levels. The mean absolute and relative weights of the spleen and thymus were not affected by the treatment. The anti-SRBC IgM serum titers were less for the 2 and 10 mg/kg treatment groups. However, the effect was not manifested in a dose-related manner (i.e., the titers for 2 and 10 mg/kg groups were 36 and 59% of the control group, respectively). These results were judged to be equivocal based on the range of variability demonstrated in the control group values and the lack of a clear dose-response. Other parameters (spleen and thymus weights, white blood cell differential counts) did not indicate any suppression of immunopotency. The positive control was functional. **Study acceptable.** (Moore, 5/3/11)

ENDOCRINE DISRUPTOR STUDIES SUPPLEMENTAL STUDIES

Human Epidemiological Studies Related to Neurotoxicity

(This is not an exhaustive list, since primary responsibility to evaluate these studies belongs to Worker Health and Safety Branch

342-543 138174 Nolan, R. J. (Study Director) "Critical analysis of the allegations of neuropathy due to chlorpyrifos submitted to the United States Environmental Protection Agency on November 7, 1994". DowElanco had identified 31 individuals for whom physicians had made at least tentative diagnoses of neuropathy having possible association with chlorpyrifos. Although several cases of massive chlorpyrifos exposure had previously been documented, only one appeared to have caused organophosphate-type delayed neuropathy (OPIDN): this was an attempted suicide in which heroic treatments were required to address severe cholinergic symptoms (investigators citing Lotti *et al.*, 1986). The primary focus of the present investigation was on OPIDN symptoms, however other neurological findings were noted where found. None of the exposures (or worst plausible estimates of exposures) were judged to have been "biologically significant" [i.e., exposures were likely to have been too low to have measurably depressed plasma ChE, or (for inhalation route) were less than the NAS guideline of 10 $\mu g/m^3$]. Studies to date have indicated that it is critical to achieve at least 50% inhibition of neurotoxic esterase in order obtain OPIDN symptoms: this is unlikely to happen except at dose sufficient to elicit major cholinergic crises. Onsets of acute symptoms in this study were compared with plausible response times for acute ChE inhibitory signs (usually within 4 hr, in any case within 24 hr). The majority of cases presented no cholinergic signs, and none presented signs which were unambiguously due to ChE inhibition. Only three persons had documented neuropathy which became evident within one month of alleged exposure (a plausible time frame for OPIDN), without a demonstrated alternate cause. Of these, no two of them had consistent symptoms. DowElanco therefore determined that the alleged neuropathologies could not reasonably be attributed to chlorpyrifos. No SB-950 worksheet is appropriate, since this is not a relevant study type, and data do not support a treatment effect. Aldous, 8/11/97.

342-707 154147 "Critical assessment of reported entitled 'Review of chlorpyrifos poisoning data'". This report was directed to Worker Health and Safety Branch for review, since the commonly expected poisoning incidents would be acute cholinergic events. No Medical Toxicology Branch review has been requested. Aldous, 8/11/97.

NON-GUIDELINE STUDIES RELATING TO CHOLINESTERASE AND METABOLISM

Human acute oral, evaluating clinical signs, metabolism, and/or cholinesterase

342-788; 168932; "A Rising Dose Toxicology Study to Determine the No-Observable-Effect-Levels (NOEL) for erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels"; (Kisicki, J.C. *et. al.*; MDS Harris, Lincoln, Nebraska; Study ID. DR K-044793-284; 4/19/99); Six male and six female human volunteers/treatment group were fasted overnight prior to being dosed orally once with 0 (placebo: lactose monohydrate), 0.5 or 1.0 mg/kg of chlorpyrifos powder (purity: 99.8%) in capsules (phase 1) or 0 or 2.0 mg/kg (phase 2) in a double blind, randomized study. The health status of each subject was monitored for up to 7 days. Vital signs (blood pressure, pulse rate, respiration rate, and body temperature) were recorded prior to dosing and at 1, 2, 4, 8, 12, 24, 48 and 168 hours after dosing. Blood samples for erythrocyte acetylcholinesterase (AChE) analysis were drawn 10 hours prior to dosing, at the time of dosing and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hours post-dose for erythrocyte AChE activity and chlorpyrifos and metabolite analyses. A blood sample was drawn prior to dosing for paraoxonase activity determination. Urine samples were collected at 12 hour intervals starting 48 hours prior to dosing and at 0 to 6 and 6 to 12 hours post-dose and 12 hour intervals thereafter up to 168 hours after dosing. Although clinical symptoms such as anorexia, diarrhea, nausea, vomiting, dizziness, dyspnea, and headache were reported, none of these signs occurred in a dose-related manner. There was no apparent treatment-related effect upon any of the vital signs. Mean erythrocyte AChE activities were not significantly affected in a dose-related manner. One subject in the 2.0 mg/kg treatment group demonstrated a maximal 30% inhibition between AChE activity reported at 0 time and at 12 hours post-dose. Otherwise, no other subject in the high dose group had a reduction in erythrocyte AChE activity greater than 12% based on the higher of the two baseline values. The blood and urine levels of chlorpyrifos and its metabolites and the paraoxonase activity analysis for individual subjects were not included in this initial report and thus could not be evaluated. **No adverse effects indicated. NOEL:** 1.0 mg/kg (based upon the 30% inhibition of erythrocyte AChE demonstrated by one of the subjects in the 2.0 mg/kg treatment group). **Supplemental Study.** (Moore, 5/18/99).

342-823 178361 This is a copy of study 342-788; 168932, above.

342-822 178360; Brzak, K. A., "A Rising Dose Toxicology Study to Determine the No-Observable-Effect- Levels (NOEL) for erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels – Part B" Acetylcholinesterase (AChE) Inhibition Study; Human; The Dow Chemical Company, Midland, MI; Laboratory I.D. No. 981176; 6/5/00; Chlorpyrifos; Human volunteers (6/sex/dose) received a single oral dose of 0.0, 0.5, 1.0 or 2.0 mg/kg (capsule form) in a double-blind clinical trial; blood and urine specimens were collected and analyzed for chlorpyrifos and its metabolites

(chlorpyrifos oxon and 3,5,6-trichloro-2-pyridinol (TCP)) using GC-MS; pretreatment Chlorpyrifos Oxonase (CPOase), paraoxonase and diazoxonase were determined spectrophotometrically; blood and urine specimens were generally below the limit of quantitation (LOQ) for chlorpyrifos; average AUC for TCP in blood (by increasing dose) was 14.0, 25.2 and 51.2 μg/g, respectively and amount TCP excreted in the urine was 4.1, 8.7 and 15.9 mg, respectively during the first 168 hr following ingestion; blood and urinary TCP levels increased rapidly, remained constant over first 48 hr post-treatment, and then declined with an average half-life of 29 to 36 hr; administration by capsule probably reduced absorption (average of 34.7%, 30.8% and 29.5% absorbed in 0.5, 1.0 or 2.0 mg/kg dose group, respectively); serum CPOase activity was within the range of activity reported in previous studies and there were no extreme values; RBC AChE depression was seen in only one individual, a 2.0 mg/kg female that showed unusually high absorption of chlorpyrifos (87.9% versus 29.5%). Supplementary Data. Kellner, 2/23/01. [NOTE by C. Aldous: This study is "Part B" of 342-788; 168932, above].

342-834 183264 This is a copy of 342-822 178360, above.

Human repeat dosing, oral, evaluating clinical signs, metabolism, and/or cholinesterase

342-0343 071392 Coulston, F., T. Griffin, and L. Golberg, "Safety evaluation of Dowco 179 in human volunteers," Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY, March 1972. Four male volunteers/group were dosed by tablet with Dowco 179 (chlorpyrifos) at 0 mg/kg/d (placebo) for 48 days, 0.014 mg/kg/d for 27 days, 0.03 mg/kg/d for 20 days, or 0.10 mg/kg/d for 9 days. Investigators assessed hematology and clinical chemistry weekly, and plasma cholinesterase (ChE) and RBC AChE twice weekly. These assessments continued as needed post-treatment to determine recovery. No treatments affected hematology or clinical chemistry or RBC AChE. Plasma ChE inhibition was marked and progressive over time at 0.10 mg/kg/d, with inhibition of 10% on days 1 to 3, 46% inhibition on day 6, and 66% inhibition on day 9, when dosing of that group was stopped. Recovery of this group progressed after cessation of dosing, with plasma ChE reaching twice the treatment day 9 activity at recovery day 11, and complete recovery to pre-treatment activity at recovery day 25. Plasma ChE activity in the 0.03 mg/kg/d group was reduced by about 30% during days 16-20. Complete recovery from this lesser effect was complete by 20 days off treatment. Study gives useful supplementary information. Aldous, June 5, 2015.

342-0607 145821 is an exact copy of 342-0343 071392, above.

Human dermal (or dermal/oral comparison), evaluating clinical signs, metabolism, and/or cholinesterase

342-122 948115 Nolan, R. J., D. L. Rick, N. L. Freshour, and J. H. Saunders, "Chlorpyrifos: pharmacokinetics in human volunteers following single oral and dermal doses," Dow Chemical, Midland, MI, Aug. 1982. Healthy male volunteers were dosed with chlorpyrifos (analytical grade, 99.8% purity) to assess kinetics of chlorpyrifos and of its major metabolite (3,5,6-trichloro-2-pyridinol), and to follow changes in plasma and RBC cholinesterase (ChE) over time. N = 5 for major parameters. Exposures were a 0.5 mg/kg single oral dose, followed 4 weeks

later (ample time for clearance from the oral exposure) by a single 5 mg/kg dermal dose. None of these doses elicited clinical signs. Following 0.5 mg/kg oral dosing, plasma ChE was inhibited to about 15% of baseline, with greatest inhibition at 0.5 to 2 hrs after dosing. By 8 hours, plasma ChE levels were 3-4 fold higher than the lowest activity. By 27 to 30 hours, plasma ChE activity was essentially back to baseline. Dermal dosing with 5 mg/kg chlorpyrifos had no definitive effect on plasma ChE at any time post-dose. RBC AChE activity was inherently more variable than plasma ChE. RBC AChE activity was not measurably affected by these oral or dermal exposure levels. Blood chlorpyrifos levels following 0.5 mg/kg oral dosing was either non-detectable, or was in the range of 5-30 ng/ml blood. The highest blood chlorpyrifos levels did not appear at consistent times post-dosing, and clearly would not represent a reliable measure of exposure. Blood concentrations of chlorpyrifos following 5 mg/kg dermal exposure were either non-detectable or did not exceed 10 ng/ml. Blood levels of 3,5,6-trichloro-2-pyridinol following 0.5 mg/kg oral dosing showed quite variable kinetics between subjects, but tended to peak at 2-8 hours at about 1 µg/ml blood, with levels at 24 hours being no less than 50% of peak concentrations. This confirms that this metabolite would be a good indicator of exposure. Dermal exposure of 5 mg/kg yielded 3,5,6-trichloro-2-pyridinol blood levels which occasionally exceeded 0.1 µg/ml. There was about a 4-fold range of peak 3,5,6-trichloro-2-pyridinol blood between dermal exposure subjects. Investigators estimated the half-life of 3,5,6-trichloro-2-pyridinol to be about 27 hours by either route. Urinary peak excretion rates of 3,5,6-trichloro-2-pyridinol were at about 9 hours for oral route, and about 42 hours for the dermal route. Time to decrease to about 50% of maximum urinary 3,5,6-trichloro-2-pyridinol levels were roughly 30 hours for oral exposure and 84 hours for dermal route. Thus this study shows that chlorpyrifos is only moderately absorbed through the skin, that plasma ChE is a good marker of systemic load for several hours after exposure, whereas urinary 3,5,6-trichloro-2-pyridinol assays would be useful for qualitative exposure assessment for 2-3 days for oral route, and slightly longer for dermal exposure. Useful supplementary data. Aldous, 4/16/15.

342-0197 001367, also 342-0627 149353 These are exact copies of 342-122 948115, above.

342-0343 071383 Nolan, R. J., D. L. Rick, N. L. Freshour, and J. H. Saunders, "Chlorpyrifos: pharmacokinetics in human volunteers," *Toxicol Appl Pharmacol* **73**, 8-15 (1984). This is a published version of Record No. 948115.

342-763 165484 Griffin, P., H. Mason, K. Heywood, and J. Crocker, "Oral and dermal absorption of chlorpyrifos: A human volunteer study", cover letter dated 11/23/98. (This was a manuscript accepted for publication in Occupational & Environmental Medicine). Data were reviewed by T. Thongsinthusak of DPR Worker Health and Safety Branch: that review is bound with the volume. Dermal applications led to 1% absorption (evidenced as dialkylphosphate urinary metabolites), and 53% unaltered chlorpyrifos was recovered by washing the application site. Investigators did not account for the balance for the remainder of residues. Aldous, 10/13/99.

Rat acute oral, evaluating clinical signs, metabolism, and/or cholinesterase

342-763 164102 Mendrala, A. L. and K. A. Brzak, "Chlorpyrifos: Part A - Concentration - time course of chlorpyrifos and chlorpyrifos-oxon in blood", The Dow Chemical Co., Midland,

8/31/98, Laboratory Project Study ID 971187A. Chlorpyrifos was administered by gavage in corn oil to male F344 rats at dose levels of 0.5 to 100 mg/kg. [Segment 1]: Four rats/group were killed at intervals of 10 min to 12 hr to determine time course of (a) concentrations of chlorpyrifos and chlorpyrifos-oxon, and (b) plasma and brain cholinesterase (ChE) activities. Chlorpyrifos concentrations peaked at 3 hr, with levels dropping substantially at 6 to 12 hr. Chlorpyrifos-oxon was only about 1% as abundant as chlorpyrifos, and was typically detectable at 1 hr and 3 hr intervals only. Plasma ChE inhibition was evident at all dose levels (15% inhibition at 0.5 mg/kg). Brain ChE inhibition was marginally evident at 5 mg/kg (NOEL = 1 mg/kg). [Segment 2]: Four rats/group were dosed by gavage in corn oil with nominal 5 or 100 mg/kg (achieved levels of 3 and 63 mg/kg) of ring-labeled ^{14}C-chlorpyrifos 3 hr prior to sacrifice. Blood was collected for measurements of circulating chlorpyrifos, chlorpyrifos-oxon, and the trichloropyridinol (TCP) hydrolysis product. TCP was by far the most abundant labeled species found in blood (about 98% of label at either dose level), with most of the remaining label as chlorpyrifos. Useful supplemental data, no DPR worksheet. Aldous, 10/13/99.

Rat chlorpyrifos acute vapor inhalation, evaluating clinical signs, metabolism, and/or cholinesterase

NOTE: The two rat acute vapor inhalation studies below assess acute responses to parent chlorpyrifos and to chlorpyrifos oxon, respectively.

342-0937; 271252; Hotchkiss, J. A., S. M. Krieger, K. M. Mahoney, K. A. Brzak, N. A. Malowinski, and D. L. Rick, "Nose-Only Inhalation of Chlorpyrifos Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Female CD(SD): Crl Rats"; (Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID No. 131040; 5/2/13); Forty female Crl:CD(SD) rats/group were exposed nose-only to either 0 (filtered air) or 17.7 ppb (0.254 µg/l) of a saturated vapor of chlorpyrifos technical (lot no. 7299412; purity: 97.6%) for 6 hours. Eight animals/group/time point were euthanized at 0, 2, 4, 6 and 12 hours post-exposure. Blood, brain and lung tissue were procured from each animal. Cholinesterase activity was assayed in the plasma, blood, brain and lungs. Blood levels of chlorpyrifos and its primary metabolite, trichloropyridinol were determined as well. The animals demonstrated no signs of toxicity during the exposure or for the 12-hour post-exposure period. The peak level of chlorpyrifos in the blood was immediately after the completion of the exposure, diminishing to a non-detectable level by 6 hours post-exposure. The trichloropyridinol peak levels were noted up to 2 hours post-exposure and gradually diminished over the 12-hour post-exposure observation period. Chlorpyrifos-oxon was not detectable in any of the samples. None of the tissues which were assayed from the exposed group demonstrated a significant reduction in cholinesterase activity in comparison to the control activity levels. Activity in the blood and plasma of the exposed animals was 93 and 86%, respectively, of the control values at 4 hours post-exposure, the maximal reduction. The ChE activity in the lungs of the exposed animals was 89% of the control group at that time point. There was no apparent effect upon ChE activity in the brain. **No adverse effect indicated. Study supplemental.** (Moore, 6/4/13)

342-0950 274123; "Nose-Only Inhalation of Chlorpyrifos-Oxon Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Female CD(SD):Crl Rats"; (J.A. Hotchkiss, S.M. Krieger,

K.M. Mahoney, K.A. Brzak, N.A. Malowinski, D.L. Rick; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID. 131067; 8/30/13); In Phase 1, the highest attainable saturated vapor concentration of chlorpyrifos-oxon (oxon) under standard laboratory conditions typical of an acute nose-only inhalation exposure study was determined and selected for Phase 2 of this study. In Phase 2, eight female CD(SD):Crl rats/group/sacrifice time were exposed for 6 consecutive hours to filtered air (control) or a time weighted average concentration of 35.3 µg/m^3 (2.58 ppb) oxon vapors using a flow-past nose-only inhalation exposure system. Rats were sacrificed immediately (0 hr) and at 1, 2, 4, 8 and 24 hours after the end of exposure. Blood and tissues were isolated and processed to determine cholinesterase (ChE) activity in red blood cells (RBC), plasma, and lung and brain tissues. Whole blood samples from n=4 rats in each group/sacrifice time were analyzed to determine the concentrations of oxon and 3,5,6-trichloro-2-pyridinol (TCP). No clinical signs of toxicity were noted in oxon-exposed rats at any time during or after exposure. No oxon was detected in the blood at any time after exposure (lower limit of quantification (LLQ), 0.118 ng/g blood), however, blood TCP levels > LLQ (2.44 ng/g blood) were detected in all assayed blood samples collected at 0 through 4 hours after exposure and in 1/4 assayed blood specimens collected 8 hours post-exposure. By contrast, blood TCP levels were below LLQ in 3/4 and 4/4 animals sacrificed at 8 and 24 hours after exposure, respectively. No oxon-induced inhibition of ChE activity was detected in RBC, plasma, lung or brain at any time after exposure. The presence of TCP in the blood of oxon-exposed rats confirms that oxon vapor is absorbed through the respiratory tract, however, the inhaled oxon is rapidly metabolized and not systemically bioavailable, given that all the assayed blood levels were below LLQ (0.118 ng/g or 3.53×10^{-4} nmol/g blood). Based on the absence of cholinesterase inhibition in RBC, plasma, brain or lung (the portal-of-entry tissue), the 6-hour No Observed Effect Concentration (NOEC) for inhaled oxon vapor is > 35 µg oxon/m^3 air. The results of this study suggest that there is no biologically relevant hazard from inhalation of a saturated vapor concentration (35.3 µg/m^3) of chlorpyrifos oxon. **Study Supplemental**. (Guo, 11/13/13)

Rat chlorpyrifos repeat-dose vapor inhalation, evaluating clinical signs, metabolism, and/or cholinesterase

342-0343 071388 Landry, T. D., D. A. Dittenber, L. L. Calhoun, L. G. Lomax, and P. Morabito, "Chlorpyrifos: 2-week nose-only vapor inhalation exposure study in Fischer 344 rats," The Dow Chemical Company, Midland, MI, 6/10/86. This study exposed female rats (N = 6) to 0 or 12 ppb chlorpyrifos vapor (99.7% purity) 6 hours/day, 5 days/week, with sacrifice one day after the last exposure (with 3 consecutive days of exposure before the day of sacrifice). Investigators evaluated cholinesterase (plasma, RBC, and brain), clinical signs, body weights, hematology, and gross pathology. There were no treatment responses. The tested concentration was noted to be about 50% of the maximum theoretical maximum vapor level for chlorpyrifos. Although individual data were provided, there is no DPR worksheet for this report, since it does not address a data requirement, and because it was negative. Aldous, 5/15/15.

342-0343 071389 Corley, R. A., T. D. Landry, L. L. Calhoun, D. A. Dittenber, and L. G. Lomax, "Chlorpyrifos: 13-week nose-only vapor inhalation exposure study in Fischer 344 rats," The Dow Chemical Company, Midland, MI, 11/13/86. This study exposed both sexes (N = 10) to 0, 5.2, 10.3, or 20.6 ppb chlorpyrifos vapor (100% purity, reporting mean assayed chamber concentrations) 6 hours/day, 5 days/week, with sacrifice one day after the last exposure (with at

least 4 consecutive days of exposure before the day of sacrifice, following overnight fasting). Investigators evaluated cholinesterase (plasma, RBC, and brain), clinical signs (shortly after each exposure period), body weights, organ weights, hematology, clinical chemistry, urinalysis, and gross pathology. Protocol tissues of both sexes were subject to histopathology examination in control and high dose groups. There were no treatment responses. The maximum vapor level for chlorpyrifos was noted to be about 25 ppb. This is a valid supplementary study. Although individual data were provided, there is no DPR worksheet for this report, since it does not address a standard data requirement, and because responses were negative. Aldous, 5/15/15.

Rat chlorpyrifos acute aerosol inhalation, evaluating clinical signs, metabolism, and/or cholinesterase

342-0908; 258214; AAcute Inhalation Exposure of Adult Crl:CD(SD) Rats to Particulate Chlorpyrifos Aerosols: Kinetics of Concentration-Dependent Cholinesterase (ChE) Inhibition in Red Blood Cells, Plasma, Brain and Lung@; (J.A. Hotchkiss, S.M. Krieger, K.A. Brzak, D.L. Rick; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID. 091133; 6/29/10); In Phase I, six Sprague-Dawley rats/sex/group were exposed nose-only to 0, 13.3 or 66.7 mg/m^3 (analytical) of Chlorpyrifos technical (lot no. KC28161419; purity: 99.8%) for six hours. Blood was drawn from an indwelling jugular catheter at 2, 4, 6 hours of exposure and at 0.5, 1, 2, 4, 6, 12, and 24 hours post-exposure. Red blood cell and plasma cholinesterase (ChE) activities were assayed for each time point. In Phase II, 54 female rats/group were exposed nose-only to 0, 3.7, 12.9, 22.1 or 53.5 mg/m^3 of the test material for up to 6 hours. Six animals/group/time point were euthanized at 2, 4, and 6 hours of exposure and at 2, 6, 12, 24, 48 and 72 hours post-exposure. Cholinesterase activities in the red blood cells, plasma, lungs and brain were assayed and the blood concentrations of chlorpyrifos (CPF), chlorpyrifos-oxon (CPF-oxon) and trichloropyridinol (TCP) were measured. Urine was collected from 6 animals/group at 0 to 12, 12 to 24, 24 to 48 and 48 to 72 hours and trichloropyridinol concentrations were determined. In Phase I, significant inhibition of red blood cell and plasma ChE activities was evident at 13.3 mg/m^3 For RCE ChE activity, maximal inhibition of 65% for males and 80% for the females was noted at 2 hours post-exposure. For plasma ChE activity, maximal inhibition of 66% for males and 87% for females was evident from 6 hours of exposure to 1 hour post-exposure. Based on these results, females were deemed to be more sensitive to the effects of CPF on ChE activity and thus were selected for testing in Phase II. ChE inhibition in the plasma achieved a maximal level of 48% at 6 hours of exposure in the 3.7 mg/m^3 group. In the lungs, a maximal level of ChE inhibition was noted at 47% in the 3.7 mg/m^3 at 6 hours of exposure. ChE activity in the brain was significantly reduced for the 12.9, 22.1 and 53.5 mg/m^3 groups with maximal inhibitions of 19, 21 and 22%, respectively, which were noted at 6, 6 and 2 hours post-exposure, respectively. For RBC AChE activity, the results were inconsistent at the 3.7 mg/m^3 exposure level possibly due to the variability of the control values. Maximal reduction in activity was not evident until 24 to 48 hours post-exposure. The blood levels of CPF were highest at 4 to 6 hours of exposure for all of the exposure levels with a peak value of 65 ng/g noted for the 53.5 mg/m^3 group. CPF-oxon was recovered in the blood at peak levels of 0.22 ng/g during the exposure at the 53.5 mg/m^3 exposure level. Peak levels of 2400 ng/g of TCP for the highest exposure group were noted at 12 hours post-exposure. The plasma half-life of CPF ranged from 0.463 to 3.34 hours over the exposure concentration range. The ratio of the areas under the curve for TCP/CPF ranged from

545 to 1057. The inhaled dose of the test material was calculated to be 1.04, 3.62, 6.21 and 15.0 mg/kg. Excretion of TCP in the urine demonstrated a half-life ranging from 10.6 to 11.6 hours. Using these excretion data the percentage of inhaled CPF which was absorbed was calculated and ranged from 36 to 79%. **Study supplemental.** (Moore, 5/2/11)

Rat chlorpyrifos life stage comparisons (as neonate vs. young adult), evaluating clinical signs, metabolism, and/or cholinesterase

342-0906; 257044; AComparison of Cholinesterase (ChE) Inhibition in Young Adult and Pre-weanling CD Rats after Acute and Repeated Chlorpyrifos or Chlorpyrifos-Oxon Exposures@; (M.S. Marty, A.K. Andrus; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID. 091107; 6/29/10); Pre-weanling (11 days post-natal) and young adult female Sprague-Dawley rats were dosed orally by gavage, using vehicles of corn oil or rat=s milk or in the diet (adult rats only) with concentrations of Chlorpyrifos technical (CPF) (lot no. KC28161419, purity 99.8%) ranging from 0.05 to 10 mg/kg, in a single dose regimen or at concentrations ranging from 0.05 to 3.5 mg/kg/d of CPF in corn oil in a 10-day multiple dosing regimen (pre-weanling: days 11 to 21 post-natal, young adult: 70 to 80 days old). Other groups of pre-weanling and young adult female rats were dosed orally by gavage in a single dose regimen with Chlorpyrifos-oxon (CPF-oxon) in corn oil (lot no. 199902031-66, purity: 94.9%) at concentrations ranging from 0.005 to 1.0 mg/kg. In a 10-day multiple dosing regimen, both pre-weanling and young adult females were dosed orally by gavage with 0.01 and 0.5 mg/kg/d of CPF-oxon in the same manner as the CPF-treated animals. Eight animals/sex were included in the pre-weanling groups and 8 females/group were dosed in the young adult cohort. Preliminary studies were performed in order to establish the time-to-peak inhibition profile for plasma, red blood cell and brain cholinesterase (ChE) inhibition. In the dose-response studies, animals were euthanized at the time-to-peak ChE inhibition. The concentrations of CPF, CPF-oxon and trichloropyridinol (TCP) in the blood of some of the study animals were determined. A functional observational battery was performed on the study animals in the multiple-dosing regimen after 9 days of dosing. The times-to-peak effect were as follows: PND 11 pups: 1. CPF in corn oil (6 hours), 2. CP0 in corn oil (4 hours), 3. CPF in rat=s milk (8 hours); young adult females: 1. CPF in corn oil (8 hours), 2. CPF-oxon in corn oil (4 hours), 3. CPF in diet (after conclusion of the 12-hour exposure period) (8 hours). Based upon the results of the dose response studies, no effect levels were established for plasma, red blood cell and brain ChE inhibition under the different dosing scenarios. In the **single dose regimen**, NOELs for the plasma and red blood cell ChE inhibition were 0.5 mg/kg for both sexes of the pre-weanlings after treatment with CPF, using either corn oil or rat=s milk as the vehicle, and for the young adult females treated by gavage, using a corn oil vehicle, or in the diet. The NOEL values for the brain ChE inhibition were 2 mg/kg for the male pre-weanlings treated with CPF, using either corn oil or rat=s milk as the vehicle, for the female pre-weanlings, using corn oil as the vehicle and for the adult females treated by gavage or in the diet. For the pre-weanling females dosed with CPF in rat=s milk, the brain ChE inhibition NOEL was 0.5 mg/kg. The NOELs for treatment with a single dose regimen of CPF-oxon were as follows: for both male and female pre-weanlings, the NOELs for plasma ChE inhibition: 0.05 mg/kg, for red blood cell ChE inhibition: 0.1 mg/kg and for brain ChE inhibition: 0.5 mg/kg. For the young adult females, the NOEL for plasma, red blood cell and brain ChE inhibition were 0.1, 0.1 and 0.5 mg/kg, respectively. In the **multiple dose regimen** in which the pre-weanlings and young adults were

treated with CPF in corn oil by gavage, the NOEL values for ChE inhibition were as follows: male and female pre-weanlings, plasma and RBC: 0.1 mg/kg, brain: 0.5 mg/kg; young adult females, plasma: 0.1 mg/kg/d, red blood cell: 0.5 mg/kg/d, brain: 0.5 mg/kg/d. The NOELs for ChE inhibition after multiple treatments with CPF-oxon in corn oil were as follows: male and female pre-weanlings and young adult females, plasma and red blood cell: 0.01 mg/kg/d, brain: 0.5 mg/kg/d. The NOEL values were reduced from 0.5 mg/kg to 0.1 mg/kg/d for plasma and red blood cell ChE inhibition in the pre-weanlings after multiple treatments with CPF in corn oil. The brain ChE inhibition for these animals was lowered from 2 mg/kg to 0.5 mg/kg/d. In the young adult females, the NOELs for plasma and brain ChE inhibition were lowered from 0.5 mg/kg to 0.1 mg/kg/d and from 2 mg/kg to 0.5 mg/kg/d, respectively. The concentrations of CPF and TCP in the blood at the NOEL and/or LOEL treatment levels for the various treatment scenarios were examined. Treatment with CPF in corn oil or rat=s milk to pre-weanling rats in either a single dose or multiple dose regimen resulted in TCP/CPF concentration ratios (based on ng/g of blood) ranging from 70 to 209. For the young female rats, in certain instances, the CPF concentration was below the limits of detection and the ratio could not be calculated. Otherwise, the ratios were 935 and 449 (0.5 and 2.0 mg/kg, by gavage, respectively), 7243 (2.0 mg/kg in the diet) in the single dose regimen and 2450 (0.5 mg/kg/d) and 651 (1.0 mg/kg/d) after multiple doses by gavage. These data indicate a possible difference in the metabolic disposition of CPF between the pre-weanling pups and the young adult animals. No treatment-related effects were identified in the FOB. **Supplemental Study.** (Moore, 2/23/11)

342-0897 253051 This is an interim report of 342-0906; 257044, above.

342-764 164103 Mattsson, J. L., J. P. Maurissen, P. J. Spencer, K. A. Brzak, and C. L. Zablotny, “Effects of chlorpyrifos administered via gavage to CD rats during gestation and lactation on plasma, erythrocyte, heart and brain cholinesterase, and analytical determination of chlorpyrifos and metabolites”, The Dow Chemical Co., Midland, 08/98. This study was not reviewed under SB-950, but has been examined extensively by R. Cochran for the chlorpyrifos risk assessment. Aldous 10/13/99.

Dog chlorpyrifos subchronic or subacute, dietary, evaluating clinical signs, metabolism, and/or cholinesterase †

342-836; 183362; “Chlorpyrifos Technical: 6-Week Dietary Study of Acetylcholinesterase Inhibition in Beagle Dogs”; (B.R. Marable, P.C. Baker, K.E. Stebbins and J.P. Maurissen; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID: 011036; 7/27/01); Four beagle dogs/sex/group received 0, 0.5, 1.0 or 2.0 mg/kg/d of Dursban FM (Chlorpyrifos Technical) (lot no. 7299412, TSN100759, purity: 97.6%) in the diet for 6 weeks. The animals were fed twice per day and the content of the AI in the diet was adjusted in a manner such that the daily intake per body weight was maintained. No deaths resulted from the treatment. There was no apparent dose-related effect upon the mean body weights. No clinical signs were noted during the treatment period. The mean red blood cell cholinesterase (ChE) activity was reduced in a dose-related manner with maximal levels of inhibition achieved after 6 weeks (% of baseline, males, 0.5: 44.5%, 1.0: 27.6%, 2.0: 14.4%; females, 0.5: 56.9%, 1.0: 32.8%, 2.0: 18.9%). There was no dose-related effect upon the brain, diaphragm, muscle or nodose ganglion acetylcholinesterase (AChE) activity for either sex after 6 weeks of treatment. The AChE activity in the left atrium of the heart of the males was reduced

in a dose-related manner (% of control, 0.5: 99.3, 1.0: 84.5%, 2.0: 74.5%). This effect was not noted for the females. **Possible adverse effect**: significant inhibition of AChE in the heart. **NOEL:** (M/F) < 0.5 mg/kg/d (based upon the reduced red blood cell ChE activity for both the males and females in the 0.5 mg/kg treatment group); **Supplemental Study** (non-guideline study) (Moore, 11/4/02)

342-833 182482 Baker, P. C. *et al.*, "Communication: Preliminary evaluation of acetylcholinesterase (AChE) in brain, peripheral tissues, and RBC in beagle dogs," The Dow Chemical Company, Midland, MI, 5/11/01. Report ID CPF0501. [Report begins on p. 38 of this volume]. Three males/group were dosed in diet with 0, 0.3, 0.6, or 1.2 mg/kg/d chlorpyrifos for 28 days. Parameters evaluated at termination focused on acetylcholinesterase measurements in RBC's, brain, nodose ganglion, left atrium, left ventricle, diaphragm muscle, and thigh muscle. In-life RBC acetylcholinesterase activity was measured weekly. All dogs survived the treatment, and there were no characteristic clinical signs. Body weight was unaffected by treatment. RBC acetylcholinesterase activity was reduced in dose-related fashion. Despite high variability in control activities, reductions in the higher two dose levels were clearly treatment-related (about 50% reduction at 1.2 mg/kg/d). These changes appeared to be progressive over time. No other tissues showed statistically significant reductions in AChE activity. Some of the assayed AChE activity values were so variable that the small numbers of dogs available could only have indicated major treatment responses. This is a useful pilot study, but data are unsuitable for quantitative analysis. Aldous, 9/27/01.

Dog chlorpyrifos subchronic or subacute, pet collar exposure, evaluating clinical signs, metabolism, and/or cholinesterase

342-244; 34080; Boyd, J. P., Cholinesterase Inhibition Study; 855; Dog; P.A.C.E. International, Dallas, TX; Project No. 20-208-1184; 5/14/85; pet collar, 8.0% AI; 6 treated animals, 4 untreated control animals; 1 collar/animal, 91 day treatment period; No mortality; Observations: no treatment-related effects, no irritation evident at the collar site; Cholinesterase (ChE) Inhibition: significant inhibition of plasma ChE from day 3 to end of study (maximal inhibition-83.7%, day 69), no apparent treatment effect on RBC AChE activity; no adverse effect; NOEL cannot be determined (significant inhibition of plasma ChE activity exhibited by treated animals); Study supplemental. (Moore, 5/12/93)

In vitro tissue studies of cholinesterase inhibition and metabolism

342-0951 274124; "*In vitro* Sensitivity of Cholinesterase to Inhibition by Chlorpyrifos-oxon in Several Tissues of the Rat"; (J.E. Chambers, E.C. Meek, H.W. Chambers; Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; Study ID. NS000128; 9/16/13); to compare the inherent sensitivity of cholinesterase in several tissues to inhibition by chlorpyrifos-oxon (CPFO) through determination of inhibitory concentrations (IC_{50} values), young adult male rats were euthanized; brain, blood, lung, heart, diaphragm, esophagus, stomach (flushed) and duodenum were removed from the animals and flash frozen in liquid nitrogen. In some animals, the heart and lungs were perfused with saline through the aorta to remove residual blood and the contents of the esophagus and duodenum were flushed out of the tissues, followed by flash freeze. Red blood cells (RBCs) collected were used intact, and also lysed and centrifuged to prepare a RBC ghost.

All tissues were homogenized (except plasma and RBC ghosts) in 0.05 M Tris-HCl buffer, pH 7.4 at 37 °C, with a motorized glass-Teflon homogenizer, and plasma was diluted and RBCs and RBC ghosts were re-suspended in this buffer. A modified Ellman (spectrophotometric) method for measurement of cholinesterase activity was used with acetylthiocholine or butyrylthiocholine (only for some of the plasma duodenum samples) as substrate and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as the chromogen. Tissue preparations were diluted in the above buffer to yield an activity level that produced about 1.2-2.0 Absorbance Units (AU) following the substrate incubation period (15 min. at 37 °C for all tissues except RBCs which was 1 hr at 37 °C) in the control samples. Five concentrations of CPFO in ethanol were used to provide an inhibition range of 20-80%; protein was quantified by the Lowry method. IC_{50} values were calculated for each of 3 replications (3 separate rats) by log-legit regression, and 95% confidence intervals were calculated for the IC_{50} means. The mean IC_{50} values (for assays conducted with acetylthiocholine as substrate, AChE) were: brain, 3.77 nM; duodenum – flushed, 3.72 nM vs. not flushed, 4.17 nM; esophagus – flushed, 3.13 nM vs. not flushed, 3.28 nM; stomach-flushed, 4.08 nM; lung – perfused, 7.21 nM vs. not perfused, 8.57 nM; heart – perfused, 3.06 nM vs. not perfused, 3.91 nM; diaphragm, 6.64 nM; RBCs, 4.19 nM vs. RBC ghosts, 5.08 nM; plasma, 55.36 nM. The assays conducted with butyrylthiocholine showed IC_{50} values very similar to those by AChE: duodenum – flushed, 3.72 nM vs. not flushed, 5.05 nM; plasma, 50.05 nM. There is no difference in the inherent sensitivity of the acetylcholinesterase in the several solid tissues studied (brain, esophagus, stomach, duodenum, heart, diaphragm, lung and red blood cells) to inhibition by chlorpyrifos-oxon, as indicated by IC_{50} values all within the same order of magnitude. The higher IC_{50} values in plasma logically result from the presence within plasma of other proteins that can be readily inhibited by CPFO (e.g., carboxylesterases) or that can absorb CPFO (e.g., albumin), thus reducing the levels of CPFO that were available to inhibit plasma cholinesterase; lower CPFO bioavailability resulted in a higher IC_{50} value, but it does not necessarily indicate lower inherent sensitivity of plasma cholinesterase. **Study Supplemental**. (Guo, 1/02/14)

342-774 165918 "Standard operating protocol for analysis of the effects of chlorpyrifos, diazinon, and sulfotep on neurite length in differentiating neuroblastoma cells in vitro." This volume is currently in evaluation by another division of DPR, and appears unlikely to be pivotal to Medical Toxicology Branch, based on its title. There are, however, studies in the public literature relating to chlorpyrifos effects on differentiating cells in culture, hence this protocol may be supportive of such a study. C. Aldous, 10/13/99.

Registrant rebuttal responses or commentaries on cholinesterase effects and inter-species extrapolations

342-790 168952 Chen, W. L., R. J. Nolan, and J. L. Mattsson, "Dow AgroSciences' response to the report of the Hazard Identification Assessment Review Committee (HIARC) entitled 'Chlorpyrifos - Hazard Identification Based on Animal Studies'". This record was an evaluation of existing data, and not a report of new data, except for an abstract of a recent human study by Kisicki *et al.* (reviewed as DPR Record No. 168932, see 1-liner below). "Laboratory Study ID" # GH-C 4904. This record was provided to call to question key US EPA conclusions regarding hazard evaluation of chlorpyrifos. **Human clinical sign evaluation:** The cited abstract concluded that the NOEL for RBC AChE was 1 mg/kg, based on 1/12 volunteers having over a 17% decrease in this enzyme at 2 mg/kg. None of the 12 volunteers at the highest dose of 2

mg/kg experienced clinical symptoms. This result suggest that a single subject presenting signs of "blurred vision, feeling of faintness, and runny nose" in an earlier study at 0.1 mg/kg/d was unlikely to have been responding to chlorpyrifos treatment. **Relevance of RBC AChE vs. BuChE:** Registrants observed that the latter has no known physiological function and no apparent relevance to human hazard assessment. In contrast, RBC AChE is evidently identical to the AChE associated with neuromuscular transmission, hence relevant in human hazard assessment. **Comparative inhibition of AChE from different sources:** Rat studies over the dose range of 10 to 100 mg/kg indicated that RBC AChE had a 12-fold lower ED_{50} than whole brain, hence regulation on blood AChE would protect against cholinergic toxicity. AChE in other tissues was less sensitive to inhibition (i.e. had a higher ED_{50}) than whole brain (p. 22). **Primary conclusions of investigators:** Investigators determined (1) that human data are valid and preferable to animal data in assessing human hazard, (2) that human RBC AChE rather than BuChE should be used to set RfD's, (3) and that the laboratory animal data base (if agencies are determined to use such for human safety assessment) is sufficiently complete that (a) there is no justification for an additional ten-fold safety factor for uncertainties regarding possible special toxicity to infants and children and (b) the comparative blood ChE responses of humans and laboratory animals (for RBC AChE and BuChE) are sufficiently well-characterized that a 10-fold interspecies uncertainty factor is not appropriate. Supportive published articles were included: (1) Chen *et al.* "Human red blood cell acetylcholinesterase inhibition as the appropriate and conservative surrogate endpoint for establishing chlorpyrifos reference dose", **Regulatory Toxicology and Pharmacology 29**, 15-22 (1999), (2) Schardein and Scialli, "The legislation of toxicologic safety factors: The Food Quality Protection Act with chlorpyrifos as a test case", **Reproductive Toxicology 13**, 1-14, 1999, and (3) Gibson, J. E. *et al.*, "How to determine if an additional 10x safety factor is needed for chemicals: A test case with chlorpyrifos", **Toxicol Sci 48**, 117-122 (1999). No worksheet (no reviewable data). Aldous, 9/14/99.

342-756 162540 Albers, J. W. *et al.*, "Determination of the reference dose for chlorpyrifos: Expert panel report." No date was given for report: cover letter date for volume was 6/19/98. Dow AgroSciences convened a panel of experts, who determined in this 85-page record that

(1) multiple studies support an RfD for repeated oral dose exposure of 0.01 mg/kg/d, and

(2) the RfD for single oral exposure was determined to be 0.05 mg/kg. There are no new studies, hence no DPR worksheet. Aldous, 10/13/99.

Mechanistic Studies on Serine Hydrolases that Degrade Endocannabinoids

The following studies by R. L. Carr *et al*. explored effects of chlorpyrifos on two serine hydrolase enzymes involved in degradation of endocannabinoid degradation: [monoacylglycerol lipase (MAGL), and fatty acid amide hydrolase (FAAH)]. The associated endocannabinoids were 2-arachidonoylglycerol (2-AG) and anandamide (AEA). The latter are essential in neurodevelopment, but their levels in CNS are controlled by the above enzymes to keep ligand concentrations at optimal levels. Test animals were male and female Sprague-Dawley rat pups, dosed with chlorpyrifos daily by gavage from PND 10 through 16 at up to 5 mg/kg/d. Tissues tested included forebrain, and sometimes midbrain and plasma. Generally cholinesterase (ChE) was assayed in parallel.

(No DPR Record or Document Number) Carr, R. L., A. L. Adams, D. R. Kepler, A. B. Ward, and M. K. Ross, "Induction of endocannabinoid levels in juvenile rat brain following developmental chlorpyrifos exposure," *Toxicol Sci* **135**(1), 193-201, 2013. Ten-day old Sprague-Dawley rat pups were dosed with chlorpyrifos (99% purity) daily by gavage in corn oil from PND 10 through 16 at 0, 1, 2.5, or 5 mg/kg/d, with groups of 6-8 (blocked by sex and litter) sacrificed at 4, 12, 24, or 48 hours after the last dose. Forebrain ChE, MAGL, and FAAH activities were assayed at these intervals, in addition to forebrain levels of the two endocannabinoids which are primarily degraded respectively by MAGL and FAAH: (2-AG and AEA). Forebrain ChE response was strongest at 12 hours after the last dose, with inhibition of 24%, 55%, and 68% at respective dose levels. ChE inhibition at 48 hours was 9%, 36%, and 46% respectively. MAGL response was strongest at 4 hours, with inhibition of 14%, 24%, and 41% at respective dose levels. MAGL inhibition at 48 hours was 7%, 16%, and 33% respectively. FAAH was more strongly inhibited: inhibition was greatest at 4 to 12 hours after the last dose. Inhibition at 12 hours was 52%, 90%, and 93% at respective dose levels. FAAH inhibition at 48 hours was 16%, 38%, and 48% respectively. Levels of 2-AG were most notably increased at 12 hours, at which time respective treated groups had elevations of 30%, 52%, and 63% over controls (all statistically significant). By 48 hours, there were no significant differences from control, however the 5 mg/kg/d group mean was 19% over control. Levels of AEA were also most notably increased at 12 hours, at which time respective treated groups had elevations of 65%, 128%, and 190% over controls (all statistically significant). By 48 hours, the only significant difference from control was at 5 mg/kg/d group (81% over control). Investigators indicated in their discussion that FAAH is the dominant degradation enzyme for AEA, evidenced by other studies showing nearly complete mitigation of AEA effects when a specific FAAH inhibitor is employed. Investigators noted further that other studies had found that 2-AG is subject to appreciable degradation by enzymes not included in the present study. Investigators concluded that particularly alteration of FAAH activity due to chlorpyrifos may alter neuronal system development at critical stages of growth. There is no DPR worksheet, as only summary data were provided. This is a valid supplementary study. Aldous, 5/13/15.

(No DPR Record or Document Number) Carr, R. L., C. A. Graves, L. C. Mangum, C. A. Nail, and M. K. Ross, "Low level chlorpyrifos exposure increases anandamide accumulation in juvenile rat brain in the absence of brain cholinesterase inhibition," *Neurotoxicology* **43**:82-89 (2014). This work is basically an extension of that described in *Toxicol Sci* **135**(1), above, assessing the lower dose of 0.5 mg/kg/d from PND 10-16, with sacrifice at 4 and 12 hours. Serum carboxylesterase was inhibited by 94% and 74% at 4 and 12 hours after the last dose, respectively. Serum cholinesterase was inhibited by 36% and 25% at 4 and 12 hours after the last dose, respectively. Forebrain cholinesterase and forebrain MAGL activities were not altered at this dose. Forebrain FAAH was reduced by 14% at 4 hours (not significant) and by 25% at 12 hours (significant, $p < 0.05$). There was no significant difference in 2-AG in forebrain at 0.5 mg/kg/d, but forebrain AEA levels were increased by 18% at 4 hours and by 37% (significant, $p < 0.05$) at 12 hours. There is no DPR worksheet, as only summary data were provided. This is a valid supplementary study. Aldous, 5/13/15.

(No Document or Record Numbers) Carr, R. L., A. Borazjani, and M. K. Ross, "Effect of developmental chlorpyrifos exposure, on endocannabinoid metabolizing enzymes, in the brain of juvenile rats," Toxicol Sci 122(1): 112-120 (2011). Male and female Sprague-Dawley rats were

exposed to 0, 1, 2.5, or 5 mg/kg/d chlorpyrifos. Most tests were performed in pups dosed on PND 10-16, with sacrifice 4 hours after the PND 16 treatment. Body weight gains were reduced (dose-related) in 2.5 to 5 mg/kg/d pups. ChE activity (as percent of control) was reduced in respective dose groups of pups by tissue as follows: forebrain (18, 41, and 52%), medulla-pons (18, 38, and 55%), and serum (32, 50, and 55%). Pup forebrain MAGL activity was reduced by 14, 22, and 37% in respective groups. Pup forebrain FAAH activity was reduced by 40, 93, and 96% in respective groups. Investigators used a fluororphosphonate-biotin (FP-biotin) probe to mark serine hydrolase enzymes in PND 16 pups and performed an SDS-PAGE separation, ultimately visualizing the marked enzymes with a chemiluminescent reagent and capturing images on x-ray film. FP-biotin probe analyses found a strong reduction of marked FAAH at 1 mg/kg/d, with no visible presence remaining at higher dose levels. MAGL staining was quite faint, even in controls, but suggested a treatment-related reduction in female pups. Another serine hydrolase enzyme, KIAA 1363, described elsewhere as highly responsive to chlorpyrifos oxon, showed a marked dose-related reduction in this treatment range. Possible importance of the latter was outside of the scope of this article, however other abstracts by Cassidy et al. indicate that spontaneous recovery of KIAA 1363 may be rapid enough to not warrant major concern. MAGL was detectible in membrane fractions but not in cytosolic fractions, when evaluated in pup brain extracts. A specific MAGL inhibitor, JZL184, reduced 2-AG hydrolysis activity to about 55% of control activity at 10 µM, with no additional inhibition at higher dose levels. This suggests that chlorpyrifos effects on MAGL are less likely to elicit profound effects on its substrate levels than effects on FAAH. Investigators concluded that chlorpyrifos inhibition of AEA hydrolysis may be the principal concern for juvenile development, with reduced FAAH enzyme activity as the most plausible cause. There is no DPR worksheet, as data are limited to summary tables and figures. Aldous, 5/14/15.

ADDITIONAL STUDIES NOT PRESENTLY ASSIGNED TO HAZARD ASSESSMENT GROUP FOR REVIEW

Record Number 275321 Epidemiology studies pertaining to chlorpyrifos exposures: considerations of reliability and utility

DPR Received Date: 12/13/2013

Study Date:

Document Number: 342-0952

Record Number 279907 Development of chemical specific adjustment factors for chlorpyrifos and chlorpyrifos oxon

DPR Received Date: 09/04/2014

Source: The Dow Chemical Company Midland, Michigan

Study Date: 10/31/2013

Document Number: 342-0960

Record Number 282730 In vitro age-dependent enzymatic metabolism of chlorpyrifos and chlorpyrifos-oxon in human hepatic microsomes and chlorpyrifos-oxon in plasma (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 281309 Chlorpyrifos reevaluation in California toxicology research in support of chlorpyrifos (pt.1-2)

DPR Received Date: 11/18/2014

Source: Dow AgroSciences Indianapolis, IN

Study Date: 11/17/2014

Document Number: 342-0964

Record Number 282735 In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282734 Age-dependent pharmacokinetic and pharmacodynamic response in preventing rats following oral exposure to the organophosphorus insecticide chlorpyrifos (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282731 The effects of plasma lipids on the pharmacokinetics of chlorpyrifos and the impact on interpretation of blood biomonitoring data (journal article)

DPR Received Date: 01/20/2015

Study Date: 02/17/2009

Document Number: 342-0965

Record Number 282729 A human life-stage physiologically based pharmacokinetic and pharmacodynamic modeling for chlorpyrifos: development and validation (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282486 Using PBPK/PD modeling for assessing the toxicity of chlorpyrifos and the risks from current and historical exposures

DPR Received Date: 01/20/2015

Study Date: 12/08/2014

Document Number: 342-0965

Record Number 282559 Chlorpyrifos PBPK/PD modeling for multiple routes of exposure

DPR Received Date: 01/20/2015

Source: Summit Toxicology, L.L.P. Allenspark, CO

Study Date: 11/08/2013

Document Number: 342-0965

Record Number 282740 Serum albumin is as efficient as paraoxonase in the detoxication of paraoxon at toxicologically relevant concentrations (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282741 Cytochrome P450-specific human PBPK/PD models for the organophosphorus pesticides: chlorpyrifos and parathion (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282653 Application of a source-to-outcome model for the assessment of health impacts from dietary exposures to insecticide residues (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282557 Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling of dermal exposure to chlorpyrifos: validation and application to mixed oral and dermal exposures

DPR Received Date: 01/20/2015

Source: Battelle Pacific Northwest Laboratories Richland, WA

Study Date: 03/05/2013

Document Number: 342-0965

Record Number 279905 A human life-stage physiologically based pharmacokinetic and pharmacodynamic model for chlorpyrifos: development and validation (journal article)

DPR Received Date: 09/04/2014

Document Number: 342-0960

Record Number 282736 A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282558 Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling of oral exposure to chlorpyrifos: impact on toxicity adjustment factors

DPR Received Date: 01/20/2015

Source: Battelle Pacific Northwest Laboratories Richland, WA

Study Date: 01/25/2013

Document Number: 342-0965

Record Number 282737 Physiologically based pharmacokinetic and pharmacodynamic model for the inhibition of acetylcholinesterase by diisopropylfluorophosphate (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282728 Chlorpyrifos PBPK/PD model for multiple routes of exposure (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282727 Development of a source-to-outcome model for dietary exposures to insecticide residues: an example using chlorpyrifos (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 274124 In vitro sensitivity of cholinesterase to inhibition by chlorpyrifos-oxon in several tissues of the rat

DPR Received Date: 10/03/2013

Document Number: 342-0951

Record Number 279906 Chlorpyrifos PBPK/PD model for multiple routes of exposure (journal article)

DPR Received Date: 09/04/2014

Document Number: 342-0960

Record Number 282738 Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282739 Human paraoxonase 1 hydrolysis of nanomolar chlorpyrifos-oxon concentrations is unaffected by phenotype or q192r genotype (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 948107) Clinical toxicity of Dursban in dog after multiple applications of aerosol formulation (18P.)

DPR Received Date:

Source: Dow Chemical U.S.A. Midland, MI

Study Date: 12/01/1968

Document Number: 342-0119

Record Number 91999) Final report on safety evaluation and metabolic studies on Dowco 179 (IN 151) (75P.) DowElanco Dowco 179

DPR Received Date: 01/08/1991

Source: Albany Medical College Experimental Pathology & Toxicology Albany, NY

Study Date: 03/01/1971

Document Number: 342-0384

Record Number 948135) Comparison of cholinesterase depression in humans and rabbits following exposure to Chlorpyrifos (22 pp.)

DPR Received Date:

Source: Dow Chemical U.S.A. Midland, MI

Study Date: 08/01/1971

Document Number: 342-0032

APPENDIX 2.

SPRAY DRIFT ESTIMATES

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M E M O R A N D U M

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FROM: Terrell Barry, Ph.D. *[original signed by T.Barry]*
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DATE: August 15, 2017

SUBJECT: Revised: Estimation of Chlorpyrifos Horizontal Deposition and Air Concentrations for California Use Scenarios

Background

This memorandum describes modeling procedures used to estimate off-site horizontal deposition and air concentrations associated with California chlorpyrifos use scenarios. The estimates produced with theses modeling procedures are suitable for use in conducting pesticide spray drift human exposure assessments. Horizontal deposition and air concentration estimates associated with primary spray drift from orchard airblast, ground boom, and aerial applications are provided.

Modeling Methods

Two computer simulation models were used in this analysis: AgDRIFT (Teske et al., 2002) and AGDISP (Teske and Curbishley, 2013). The United States Environmental Protection Agency (US EPA) Office of Pesticide Programs (OPP) uses AgDRIFT for all agricultural deposition analysis and uses AGDISP for mosquito adulticide application scenarios (US EPA, 2014 and 2013a). For the analysis presented in this document, the AgDRIFT 2.0.05 model was used to produce the ground boom and orchard airblast deposition estimates only and AGDISP 8.28 was used to produce all aerial application deposition and air concentration estimates.

For this analysis, the AgDRIFT model was chosen for orchard airblast and ground boom because it is the only accepted model available for these two application scenarios. The AGDISP 8.28 model includes a ground boom algorithm, but that algorithm is still under development.

AgDRIFT estimates horizontal deposition for orchard airblast and ground boom applications using empirical models. The data on which the AgDRIFT empirical models are based were produced by the Spray Drift Task Force (SDTF) and were reviewed in a formal peer review (https://archive.epa.gov/scipoly/sap/meetings/web/html/121097_mtg.html). That peer review led to the current grouping of orchard types and ground boom scenarios. AgDRIFT version 2.0.05 executable file dated 8/2002 was used for all orchard airblast and ground boom simulations in this memorandum. AgDRIFT 2.0.05 is an older version of the model but produces ground boom and orchard airblast deposition results identical to the current regulatory version AgDRIFT 2.1.1. In addition, the 90th percentile ground boom results obtained from AgDRIFT 2.0.05 were identical to deposition results shown in the USEPA guidance on spray drift (White et al., 2013) that USEPA produced using the regulatory version of AgDRIFT 2.1.1. The regulatory version of AgDRIFT 2.1.1 was not available when the analysis presented in this memorandum was conducted.

The AGDISP 8.28 model was used for aerial application deposition and air concentration estimates reported in this memorandum. AGDISP is a well vetted model developed through the work of NASA, USDA Forest Service, and the US Army (Bird, et al., 2002). It is a Lagrangian first principles model that is in the public domain and has a Gaussian handoff module to estimate spray drift beyond 2605 ft. The AGDISP model has ongoing support from partnerships between various government agencies and private sector entities and is under continual improvement to bring the model behavior more accurately into line with field measured data. The AgDRIFT model contains an older version of the AGDISP aerial algorithms incorporated to estimate aerial application spray drift. However, the AgDRIFT model is limited to 2605 ft. In addition, AgDRIFT is a proprietary model developed by the SDTF in cooperation with USEPA Office of Research and Development (ORD) under a Cooperative Research Agreement (CRADA). AgDRIFT 2.1.1 does not include a time step improvement incorporated into AGDISP 8.28 (M. Teske, pers. comm., 2014). The lack of that time step improvement in AgDRIFT 2.1.1 results in higher off-site deposition relative to AGDISP 8.28. Analysis later in this memorandum shows that the regulatory version of AgDRIFT 2.1.1 does produce deposition results greater than AGDISP 8.28.

Development of Exposure Scenarios

The deposition and air concentration estimates presented in this document were developed to reflect off-site movement expected under California chlorpyrifos use patterns. Key California use scenario patterns were selected for this analysis (Table 1). A range of application sizes were produced for each of the use scenarios was chosen based upon US EPA default (US EPA, 2013a) and/or analysis of the Pesticide Use Report (PUR) (Tuli, 2013). For orchard airblast the largest application is 40 acres, for ground boom the largest application is 300 acres, for aerial the largest acreage for tree fruit and nuts is 350 acres and for high acreage field crops the highest acreage is

900 acres. A preliminary screening deposition of 0.35% of the application rate was used for initial drift model scenario scoping (S. Beauvais, pers. comm., 2014). This preliminary screening deposition was used only to rank aircraft according to the distance downwind to the deposition fraction of 0.35%. The fixed wing and rotary aircraft showing the longest distance to 0.35% were then chosen to estimate exposures due to horizontal deposition and air concentrations. This process is described in more detail below.

Table 1. Application type scenarios for chlorpyrifos deposition estimates (all application methods) and chlorpyrifos air concentration estimates (aerial application methods only).

Application type	Sub-Type
Orchard Airblast	Sparse/Young
	Dormant Apple
	Vineyard
Ground Boom Medium/Coarse	Low Boom (20 in above the canopy)
	High Boom (50 in above the canopy)
Aerial	Fixed Wing
	Helicopter

The SDTF orchard airblast data is categorized into 5 composite orchard types. The sparse/young orchard airblast is the average of small grapefruit and dormant apple orchards field data. Small grapefruit trees are young, short trees. Dormant apple consists of field data only for apple orchards without leaves. The dormant apple orchard type is based only on the field data for dormant apples. The orchard airblast and ground boom scenarios models are empirical fits to the SDTF field trial data. There are no input variables beyond the orchard type for orchard airblast or spray quality (droplet spectra) and boom height for ground boom. For example, weather conditions cannot be changed. The empirical model outputs reflect the weather conditions at the time of the field trials. For orchard airblast, the only orchard type affected by wind speed was dormant apples where the wind speeds for the field trials varied between 4 mph and 12 mph (SDTF, 1997a). The ground boom field trials were conducted near Plainview, Texas. The weather during the field trials covered a wide range of conditions. The ground boom medium/coarse field trials showed environmental conditions spanning 5 mph to 20 mph wind speeds, 44º F to 91º F air temperatures, and 8% to 82% relative humidity (SDTF, 1997b).

The aerial application model algorithm in both AgDRIFT and AGDISP is a Lagrangian model that tracks droplets released from the nozzles during the simulated application. This type of

model is called a first principles model because the deposition and air concentration estimates are obtained using the laws of physics rather than through statistical fit to observed data. Thus, the aerial model allows input of a wide range of important aspects of an aerial application. Choice of aircraft, how that aircraft is configured, and the specifications of how an aerial application is conducted can make a significant difference in the degree of off-site movement. It is important that the aerial application scenarios simulated are representative of the expected use patterns and that the inputs are clearly stated. For this analysis aerial application information obtained by the Enforcement Branch was used to select candidate aircraft and meteorological conditions (R. Sarracino, pers. comm., 2014). The AGDISP model has a large aircraft library that can be accessed to insure that each aircraft is correctly specified in the model runs. The aircraft list obtained from the Enforcement Branch was examined to match with aircraft that were in the AGDISP aircraft library. All aircraft on the Enforcement Branch aircraft list that were in the AGDISP aircraft library were used for the exploratory analysis and are shown in Table 2. For the exploratory analysis, the meteorological inputs were chosen to reflect an early summer morning application in the San Joaquin Valley. The specific meteorological inputs were the mean wind speed, temperature, and humidity for the time of 0600 hrs over 5 years of weather data (2009-2013) for the dates June 1 to August 31 from the Fresno State CIMIS weather station (station #80). Table 2 shows, for each of the candidate aircraft, the distance to 0.35% horizontal deposition of application rate. Based upon the greatest distance to the preliminary screening deposition level of 0.35% of application rate (S. Beauvais, personal communication, January 29, 2014) the AT802A fixed wing and the Bell 205 helicopter were chosen for further refinement in the final modeling scenarios.

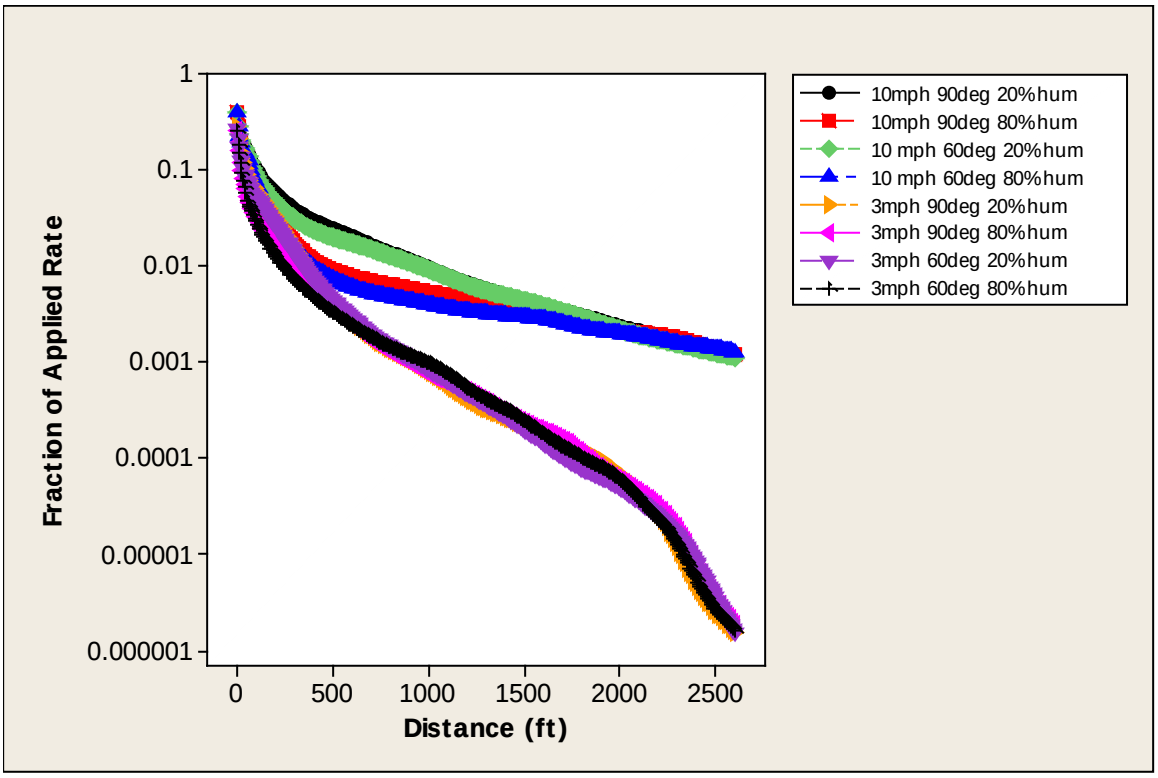
Table 2. Candidate aircraft. All simulations were conducted with a boom length of 76.3% of semi-span or rotor diameter, swath width of 60ft for fixed wing or 1.2x rotor diameter for helicopter, a swath-displacement of 37%, no half-boom effect or swath offset, 2 gal/ac volume, non-volatile active ingredient application rate of 2 lb/ac, 10 mph wind, air temperature 65 deg F, and humidity of 50%. Number of nozzles for each aircraft is the default in the AGDISP library.

Aircraft	Distance to 0.35% of application rate (ft)	Air Speed (mph)	Aircraft Weight (lbs)	Semi-span or Rotor Radius (ft)	Number of Nozzles
Fixed Wing					
AT802A	1174	145	11160	29	39
AT401	1122	120	6000	24.5	42
Trush	1102	140	7665	23.75	32
AT502	1096	155	6660	25	34
AT301	1037	120	5600	22.6	30
AgCat*	1437	150	5022	21.25	29
Helicopter					
Bell 205	1122	92	7697	24	32
Bell 47G-3B-2	1056	58	2422	18.6	25
Hiller UH-12E3	1056	58	2430	17.7	24
Hiller UH-12E3T	1056	58	2370	17.7	24
Aerodyne Wasp	1050	62	2090	17.4	24
Bell 206 Jet Ranger II	1037	69	2053	16.7	23
Bell 206 Jet Ranger III	1037	69	2398	16.7	23
Robinson R-44 Raven	1037	130	1829	16.5	22

*Biplane

Once the AT802A and the Bell 205 aircraft were chosen, the weather conditions were refined for potential worst case conditions. The information gathered by the Enforcement Branch indicated that late afternoon summer applications were expected (R. Sarracino, pers. comm., 2014). Thus, range of weather conditions were chosen to span the possible conditions from sunrise to late afternoon. AGDISP model runs were conducted using all combinations of weather conditions as follows: winds speed 3 mph and 10 mph, temperature 60 deg F and 90 deg F, humidity 20% and 80%. A total of 8 combinations of the chosen wind speed, temperature, and humidity values were simulated for the AT802A aircraft to determine the reasonable worst case weather scenario. The reasonable worst case weather scenario was then used to produce both the deposition and air concentration estimates for the AT802A and the Bell 205 aircrafts. Figure 1 shows the deposition results from those 8 model runs. The 10 mph/20% humidity model runs show the overall highest deposition. The 10 mph/20%humidity/90 deg F scenario shows generally the higher deposition than the 10mph/20% humidity/60 deg F scenario. Thus, the 10 mph/20%humidity/90 deg F meteorology combination was used to produce the deposition and the accompanying air concentrations for the AT802A and the Bell 205 application method scenarios.

Figure 1. AGDISP estimated deposition for the AT802A aircraft under 8 combinations of wind speed, temperature, and humidity.



Uncertainty

No uncertainty factors were added to the modeled deposition or the air concentration estimates. Reasoning for the three application methods of aerial, orchard airblast and ground will be considered separately.

Orchard Airblast. The AgDRIFT orchard airblast empirical model outputs the value of the empirical function. In the case of the least squares fit empirical function this value is the 50th percentile deposition estimate for three orchard types: normal, dense, and sparse. Sparse orchard type was used for this analysis to generally represent California orchards during the dormant spray season, which is reasonable worst case for near field deposition. A refined estimate for specific orchard types is also available. The dormant apples orchard type was simulated as a California specific scenario. The AgDRIFT user manual does not state why a 90th percentile is not estimated for the orchard airblast empirical equations. At the 1999 SAP OPP staff did present tolerance bounds for orchard airblast (U.S. EPA, 1999) but these bounds were not implemented.

Ground Boom. The AgDRIFT ground boom empirical model outputs the value of the empirical function. In the case of the least squares fit empirical function this value is the 50th percentile deposition estimate. In addition, the AgDRIFT ground boom empirical model has the choice to output 90th percentile. However, the derivation of the 90th percentile is not clear. This estimated deposition value does not appear to be large enough, compared to the mean at each distance, to be a tolerance interval capturing the 90th percentile at each distance with a 90% or 95% confidence. More likely what is labeled as the 90th percentile is actually the 90% prediction interval on the empirical function. There is no information provided in the AgDRIFT user manual about exactly how 90th percentile was derived. In the absence of the details of this estimate, and to maintain uniformity in approach between orchard airblast and ground boom, it is preferable to use the 50th percentile estimate (the value on the deposition curve).

Aerial. The AGDISP model produces an ensemble average deposition at a particular distance. For aerial applications all input variables were reasonable worst case. Thus, with all inputs selected for reasonable worst case, the results can be argued to represent a reasonable upper bound on the mean deposition. The AGDISP model algorithm has been compared to numerous field studies and found to produce estimates that are within a factor of two to six of field measured deposition (Bird et al., 2002; Teske and Thistle, 2003; Teske et al., 2003). The AGDISP model algorithm has been found to over-predict deposition in the far field (Bird, et al., 2002). The AGDISP air concentrations estimates have not been compared to field data. However, as mentioned earlier, AGDISP is a first principles model. In addition, mass balance is a feature of the model (Teske and Curbishley, 2013). The air concentration estimated at a particular location includes all the mass in the vertical plane at that location that is present after deposition. Thus, it is likely that the air concentrations will not be sustainably underestimated.

Deposition Estimate Development

Number of swaths. The AgDRIFT and AGDISP models have a maximum number of swaths for each application type. Application sizes are not specified. Instead, the downwind deposition reflects the number of upwind swaths. For these simulations it is assumed that the wind direction is perpendicular to the swath direction and that the deposition estimated is the deposition expected directly downwind from the middle of the swath. Thus, application size was modeled based upon the width in feet of a particular number of swaths. It was further assumed that the field to which the application was made is square. So, the width of the field and the length of the field are assumed to be equal (for aerial applications swath displacement is not considered). The acreage is calculated as the length times the width. For all three application types (orchard airblast, ground boom, and aerial), the width of the desired maximum acreage exceeded the width of the maximum number of swaths the model can simulate. For orchard airblast and

ground boom a maximum of 20 swaths can be simulated. For aerial applications a maximum of 50 swaths can be simulated. Table 3 shows a summary of swath width, maximum number of swaths and the resulting maximum acreage the model will directly produce for each application type.

Table 3. Swath parameter and limits in the AgDRIFT and AGDISP models.

Application Type	Swath Width	Max Number of Swaths	Width of Max Number of Swaths	Equivalent Square Acreage
Orchard Airblast	16 ft	20	320 ft	2.35 ac
Ground Boom	45 ft	20	900 ft	18.6 ac
Aerial Fixed-wing AT802A	60 ft	50	3000 ft	206.6 ac
Aerial Helicopter Bell 205	57.6 ft	50	2880 ft	190.4 ac

The PUR analysis indicates that use patterns in California for orchard airblast and ground boom are commonly much larger than the maximum 20 swath simulations available out of the AgDRIFT model. In order to obtain deposition estimates for applications larger than the maximum single model run limit of 20 swaths the deposition curves from one or more single 20 swath applications were overlaid after being offset upwind by the appropriate distance. Table 4 and Figure 1 show the process for orchard airblast. For orchard airblast, the AgDRIFT model estimates deposition to a maximum downwind distance of 997.4 ft (the prediction domain of the model). A model run of the maximum number of 20 swaths, assuming that rows of the orchard are 16 ft apart (16 ft wide), represents an orchard that is 320 ft wide (20 swaths × 16 ft). With the assumption of a square orchard (320 ft × 320 ft) this results in an orchard that is 2.35 ac. If a second set of 20 swaths is added to the upwind side of this initial orchard then the resulting orchard is 40 swaths, or 640 ft, wide. A square 640 ft by 640 ft orchard is 9.4 ac. Although assuming the next size up orchard is twice as wide and twice as long may seem arbitrary, for the purposes of estimating drift that assumption is not critical because only the width in the upwind direction is most important in determining the downwind deposition. The square orchard is a simplifying assumption. The grape vineyard scenario did not require extension beyond one set of 20 swaths (Table 5). The same extension procedure is used to increase the ground boom application size. Details of the ground boom process are shown in Table 6.

Table 4. Orchard airblast swath extension details. Each set of 20 swaths is 320 ft wide. Downwind deposition curves are offset by the appropriate number of feet and then overlaid. When overlaying, upwind deposition curves are allowed to drop to zero at the model domain limit of 997.4 ft.

Swath Set	Swath Width (ft)	Number of Swaths	Total Application Area Width (Sum of Set Widths)	Upwind Offset (ft)	Total Number of Swaths	Resulting Application Size (acres)	Deposition Curve Distance at Set 1 Downwind Edge (ft)	Section of Deposition Curve added to Set 1 Deposition Curve (ft)
1	16 ft	20	320 ft	0 ft	20	2.35 ac	0 ft	0 ft to 997.4 ft
2	16 ft	20	640 ft	320 ft	40	9.4 ac	320 ft	320 ft to 997.4 ft
3	16 ft	20	960 ft	640 ft	60	21.2 ac	640 ft	640 ft to 997.4 ft
4*	16 ft	20	1280 ft	960 ft	80	37.6 ac	960 ft	960 ft to 997.4 ft

*Set 4 is too far up wind to reliably estimate residue contributions to the downwind deposition curve.

Table 5. Grape Vineyard. Conventional and wrap-around sprayers. Each set of 20 swaths is 240 ft wide. Downwind deposition curves for these scenarios are not overlaid with additional upwind blocks because the deposition is so low that overlays are not necessary.

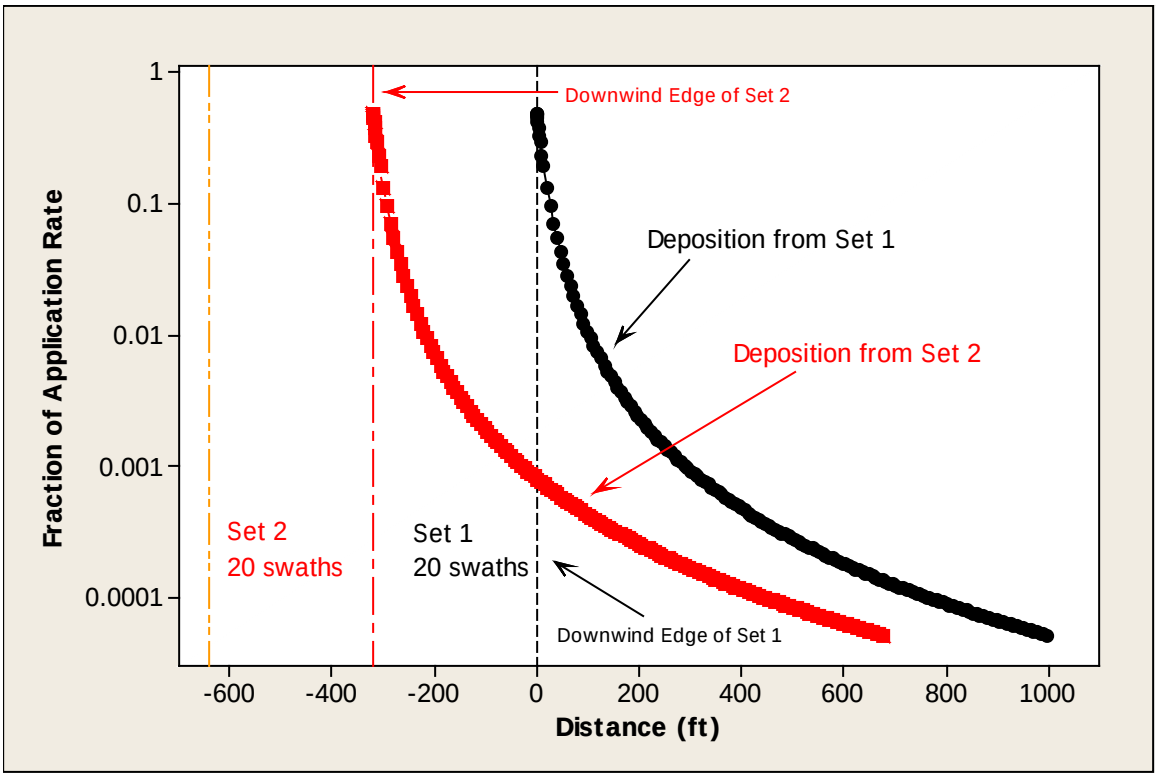
Set	Swath Width (ft)	Number of Swaths	Total Application Area Width (Sum of Set Widths)	Upwind Offset (ft)	Total Number of Swaths	Resulting Application Size (acres)	Deposition Curve Distance at Set 1 Downwind Edge (ft)	Section of Deposition Curve added to Set 1 Deposition Curve (ft)
1	12 ft	20	240 ft	0 ft	20	1.32 ac	0 ft	0 ft to 997.4 ft

Table 6. Ground boom. Each set of 20 swaths is 900 ft wide. Downwind deposition curves are offset by the appropriate number of feet and then overlaid. When overlaying, upwind deposition curves are allowed to drop to zero at the model domain limit of 997.4 ft.

Set	Swath Width (ft)	Number of Swaths	Total Application Area Width (Sum of Set Widths)	Upwind Offset (ft)	Total Number of Swaths	Resulting Application Size (acres)	Deposition Curve Distance at Set 1 Downwind Edge (ft)	Section of Deposition Curve added to Set 1 Deposition Curve (ft)
1	45 ft	20	900 ft	0 ft	20	18.6 ac	0 ft	0 ft to 997.4 ft
2	45 ft	20	1800 ft	900 ft	40	74.4 ac	900 ft	900 ft to 997.4 ft

As an example, the deposition curves from two sets of 20 swaths (Set 1 and Set 2) are overlaid to estimate the composite deposition from the 40 swaths (the total deposition resulting from joining two sets of 20 swaths). The deposition curve from Set 2 is constrained to be used only to 997.4 ft relative to the downwind edge of set 2 (Figure 2). Thus, residues from the Set 2 set of 20 swaths contribute to the downwind deposition from the orchard (Set 1 + Set 2) as a whole only between 0 ft and 677.4 ft on the deposition curve of the Set 1 set of 20 swaths. This process can be repeated for multiple sets of 20 swaths until the upwind setback is so large that the farthest upwind deposition curve extending beyond the downwind edge of the initial set of 20 swaths has a portion too small to sufficiently estimate the residues from the upwind set of swaths. For example, Set 4 in the orchard airblast scenario is too far up wind to reliably estimate residues from Set 4 that might be deposited downwind of Set 1.

Figure 2. Illustration of the deposition curve overlay process to obtain a composite deposition curve for a 40 swath orchard. Two separate 20 swath deposition curves are overlaid as shown below. The Set 2 (red deposition curve) residues only contribute to the total downwind deposition beyond the downwind edge of Set 1. The Set 2 deposition curve is not extended beyond 997.4 ft relative to the downwind edge of Set 2. So, the portion of the composite deposition curve between 667.4 ft and 997.4 ft the Set 1 downwind edge does not receive any deposition from Set 2. This is illustrated by the end of the red deposition curve.



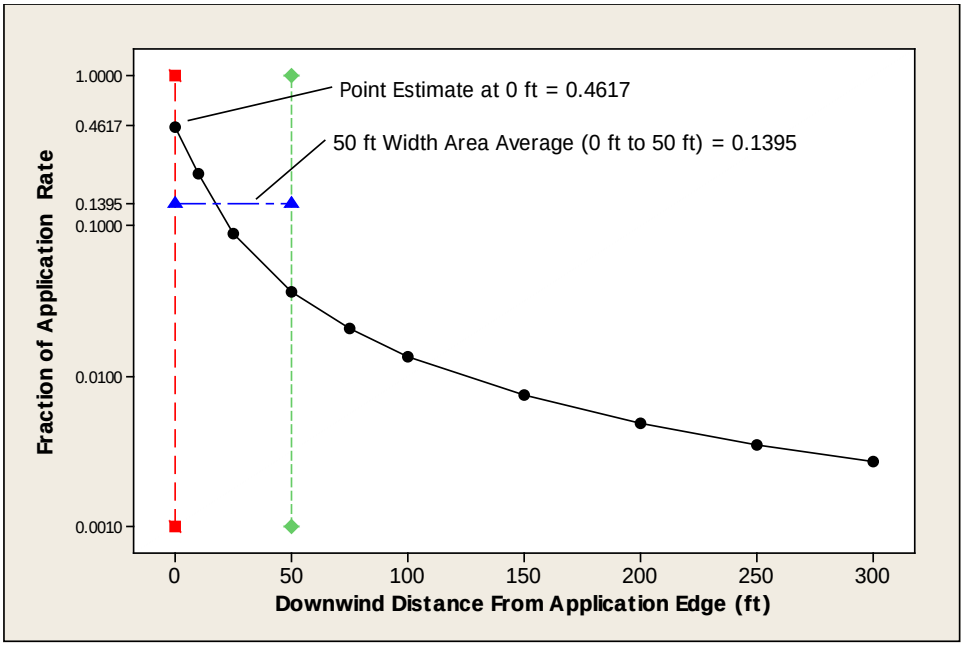
As stated above, this procedure was only implemented if the resulting deposition from the offset upwind swaths was within the prediction domain of the model. The aerial algorithm estimates deposition up to 2605 ft directly downwind of the application (the far field Gaussian handoff was not used in this analysis). The width of the first 50 swaths is 3000 ft for the fixed-wing and 2880 ft for the helicopter. So, the deposition curve from a second set of 50 swaths would fully land on the area of the application comprised by the first 50 swaths. Essentially, all of the deposition from the second set of 50 swaths lands on target. Thus, no new residue would be added to the downwind deposition curve of the first 50 swaths. For this reason the deposition curve overlay procedure was not used for aerial applications. The aerial results were obtained directly out of the AGDISP model.

Once the appropriate composite deposition curves were assembled for 40 swaths and 60 swaths, the point estimates and 50 ft width average deposition at desired distances were produced by fitting an empirical function using TableCurve 2D (AISN, 2000). The purpose of this curve fit was strictly to faithfully reproduce the modelled deposition curve, not as an explanatory analysis. This provided a convenient way to find the deposition at any desired downwind distance. All composite deposition curves were fit in TableCurve2D. Deposition estimates for orchard airblast and ground boom start at 25 ft from the downwind application edge. The SDTF field studies on which the empirical models are based did not include any sampling closer than 25 ft. Thus, the AgDRIFT empirical equations between the field edge and 25 feet are an estimation based on the assumed empirical functions for each of the application methods. These assumed empirical functions may be correct, however, with the data currently available it is impossible to verify that they reflect the actual pattern of deposition very close to the field edge. The deposition fraction likely changes rapidly close to the field. Thus, without measurements it is difficult to place confidence in the empirical estimates between 0 ft and 25 ft. For the ground boom model, the AgDRIFT manual (Teske et al., 2002) shows that a segmented approach is used to produce deposition estimates with two separate functions for 0ft to 25 ft and greater than 25 ft. The orchard airblast does not include a segmented function but the same concerns apply. Reliability of the empirical fit in the downwind direction is also a concern but the empirical functions in the far field decrease slowly and more likely over estimate deposition rather than underestimate. The AgDRIFT manual includes a detailed discussion of far field deposition distances (Teske, et al., 2002). The aerial algorithm is a first principles physics based model so estimates closer than 25 ft are provided.

Two types of estimates were provided, point estimate and an average estimate over a 50 ft width. The 50 ft width is the USEPA standard lawn scenario (USEPA, 2013b). Figure 3 compares the point estimates to the 50ft width area average. This is a generic example not related to chlorpyrifos specifically. The Average Area Deposition is calculated by integrating the area under the deposition curve between a starting downwind distance and a desired width and then dividing by the width. For example, as shown in Figure 3, integrating between 0 ft and 50 ft and

then dividing by 50 ft. In essence this spreads the area under the curve evenly between 0 ft and 50 ft. The difference between the point estimate and the area average is greatest near the application edge because the deposition curve is steep near the application edge (the slope of the curve is steeply negative).

Figure 3. Illustration of the 50 ft Width Average Deposition calculation. The 50 ft width is a moving 50 ft wide segment that depends on the starting downwind distance. In this illustration the starting downwind distance is 0 ft (the application edge) and the segment extends to 50 ft downwind. However, the process is the same regardless of the start and end point of the interval or the width of the interval. See the text for calculation details.



Deposition Estimates

Deposition estimates at selected distances for each scenario are shown in this section. The 20 swath estimates are output directly from either the AgDRIFT or AGDISP model. As described above, all 40 swath and 60 swath estimates are obtained by fitting a function to closely replicate the overlaid deposition curves (R^2 > 99.9%). The 40 swath and 60 swath point and 50ft width average deposition at the selected distances was then evaluated in TableCurve 2D.

Orchard Airblast. Sparse orchard (Tables 7 to 9), dormant apples (Tables 10 to 12), and grapevines (Tables 13 and 14) were simulated. The AgDrift sparse orchard scenario combines

the deposition results from young grapefruit and dormant apples. Dormant apples show higher deposition than sparse orchards near field but lower deposition in the far field (Figure 4).

Table 7. Sparse orchard 20 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of App	2 lb/ac µg/cm^2		Start	End	Fraction of App	2 lb/ac µg/cm^2
25	0.10070	2.2574		25	75	0.04430	0.9931
50	0.03730	0.8362		50	100	0.02000	0.4483
75	0.01810	0.4057		75	125	0.01100	0.2466
100	0.01030	0.2309		100	150	0.00680	0.1524
150	0.00440	0.0986		150	200	0.00320	0.0717
200	0.00230	0.0516		200	250	0.00180	0.0404
250	0.00140	0.0314		250	300	0.00110	0.0247
300	0.00090	0.0202		300	350	0.00080	0.0179

Table 8. Sparse orchard 40 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.10138	2.2726		25	75	0.04472	1.0025
50	0.03783	0.8480		50	100	0.02033	0.4558
75	0.01850	0.4147		75	125	0.01142	0.2560
100	0.01078	0.2418		100	150	0.00729	0.1635
150	0.00492	0.1103		150	200	0.00371	0.0831
200	0.00279	0.0626		200	250	0.00224	0.0502
250	0.00180	0.0403		250	300	0.00150	0.0336
300	0.00125	0.0280		300	350	0.00107	0.0240

Table 9. Sparse orchard 60 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.10151	2.2756		25	75	0.04488	1.0060
50	0.03799	0.8517		50	100	0.02044	0.4581
75	0.01860	0.4169		75	125	0.01148	0.2574
100	0.01085	0.2431		100	150	0.00733	0.1644
150	0.00495	0.1110		150	200	0.00373	0.0836
200	0.00281	0.0630		200	250	0.00225	0.0505
250	0.00181	0.0405		250	300	0.00151	0.0338
300	0.00126	0.0282		300	350	0.00108	0.0242

Table 10. Dormant apples 20 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.14380	3.2236		25	75	0.05520	1.2374
50	0.04350	0.9751		50	100	0.02090	0.4685
75	0.01820	0.4080		75	125	0.01010	0.2264
100	0.00930	0.2085		100	150	0.00560	0.1255
150	0.00330	0.0740		150	200	0.00230	0.0516
200	0.00160	0.0359		200	250	0.00120	0.0269
250	0.00090	0.0202		250	300	0.00070	0.0157
300	0.00050	0.0112		300	350	0.00040	0.0090

Table 11. Dormant apples 40 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.14416	3.2317		25	75	0.05530	1.2397
50	0.04380	0.9818		50	100	0.02101	0.4711
75	0.01846	0.4139		75	125	0.01028	0.2305
100	0.00948	0.2125		100	150	0.00583	0.1306
150	0.00350	0.0784		150	200	0.00244	0.0548
200	0.00169	0.0379		200	250	0.00128	0.0288
250	0.00097	0.0217		250	300	0.00077	0.0173
300	0.00061	0.0136		300	350	0.00049	0.0111

Table 12. Dormant apples 60 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.14422	3.2330		25	75	0.05535	1.2409
50	0.04385	0.9830		50	100	0.02106	0.4721
75	0.01851	0.4150		75	125	0.01033	0.2315
100	0.00952	0.2135		100	150	0.00587	0.1315
150	0.00353	0.0792		150	200	0.00248	0.0555
200	0.00172	0.0386		200	250	0.00131	0.0294
250	0.00099	0.0223		250	300	0.00079	0.0178
300	0.00063	0.0141		300	350	0.00051	0.0115

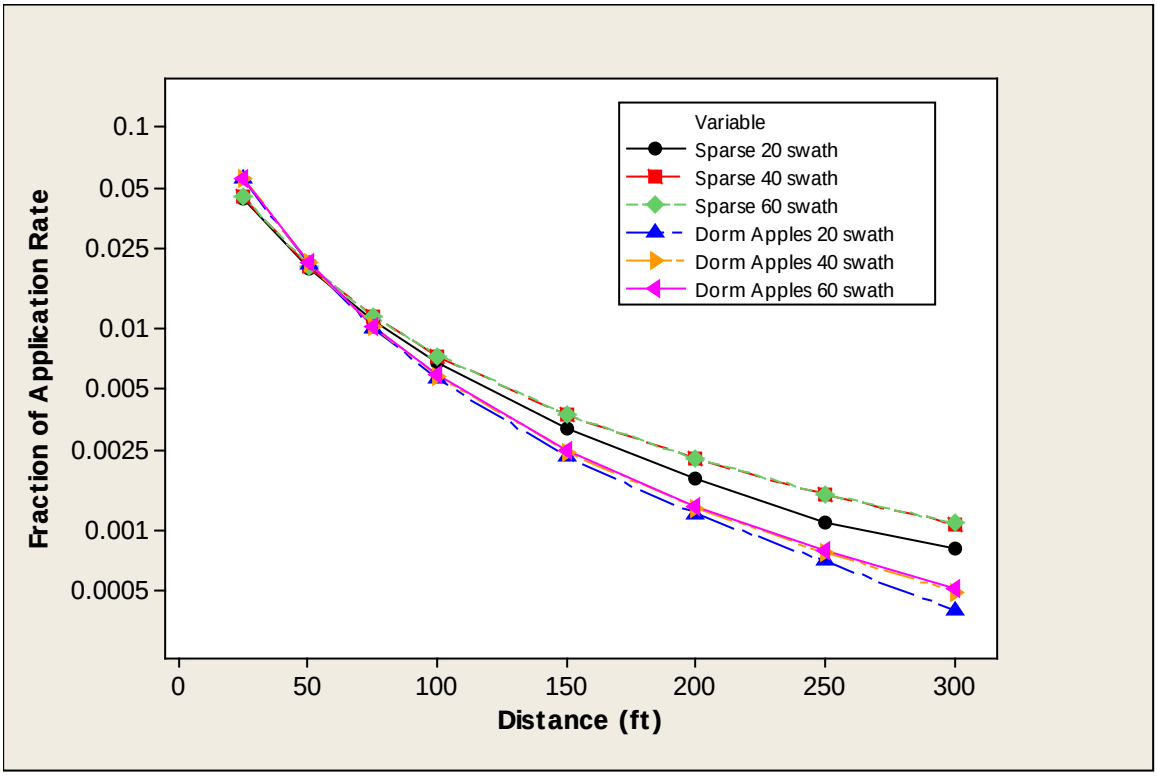
Table 13. Grape vineyard conventional sprayer 20 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.0047	0.10000		25	75	0.0022	0.04960
50	0.0019	0.04290		50	100	0.0012	0.02660
75	0.0011	0.02500		75	125	0.0008	0.01770
100	0.0008	0.01710		100	150	0.0006	0.01300
150	0.0004	0.01000		150	200	0.0004	0.00828
200	0.0003	0.00687		200	250	0.0003	0.00592
250	0.0002	0.00511		250	300	0.0002	0.00451
300	0.0002	0.00399		300	350	0.0002	0.00359

Table 14. Grape vineyard wrap-around sprayer 20 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.0007	0.01620		25	75	0.0004	0.00971
50	0.0004	0.00902		50	100	0.0003	0.00646
75	0.0003	0.00624		75	125	0.0002	0.00487
100	0.0002	0.00478		100	150	0.0002	0.00392
150	0.0001	0.00325		150	200	0.0001	0.00283
200	0.0001	0.00247		200	250	0.0000	0.00221
250	0.00009	0.00199		250	300	0.0000	0.00182
300	0.00007	0.00166		300	350	0.0000	0.00154

Figure 4. Orchard airblast application 50 ft width average deposition. Comparison between sparse orchard and dormant apples. The development procedure for these deposition estimates is described in the text.



Ground Boom. Low boom (Tables 15 and 16) and high boom (Tables 17 and 18) applications were simulated. A comparison of all deposition estimates is shown in Figure 5. As expected, high boom shows higher deposition than low boom both in the near field and the far field. The 40 swath applications show only slightly higher deposition than the 20 swath applications. This is expected because the 20 swath application is 900 feet wide, only 97 feet less than the domain of the Set 2 deposition curve.

Table 15. Ground boom deposition. Low boom and medium/coarse spray quality 20 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.0083	0.1861		25	75	0.0047	0.1054
50	0.0043	0.0964		50	100	0.0032	0.0717
75	0.0031	0.0695		75	125	0.0024	0.0538
100	0.0024	0.0538		100	150	0.0020	0.0448
150	0.0017	0.0381		150	200	0.0015	0.0336
200	0.0013	0.0291		200	250	0.0012	0.0269
250	0.0011	0.0247		250	300	0.0010	0.0224
300	0.0009	0.0202		300	350	0.0009	0.0202

Table 16. Ground boom deposition. Low boom and medium/coarse spray quality 40 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.0085	0.1898		25	75	0.0050	0.1119
50	0.0046	0.1029		50	100	0.0034	0.0767
75	0.0034	0.0753		75	125	0.0026	0.0582
100	0.0026	0.0573		100	150	0.0020	0.0459
150	0.0017	0.0381		150	200	0.0015	0.0340
200	0.0014	0.0304		200	250	0.0012	0.0274
250	0.0011	0.0247		250	300	0.0010	0.0228
300	0.0009	0.0212		300	350	0.0009	0.0197

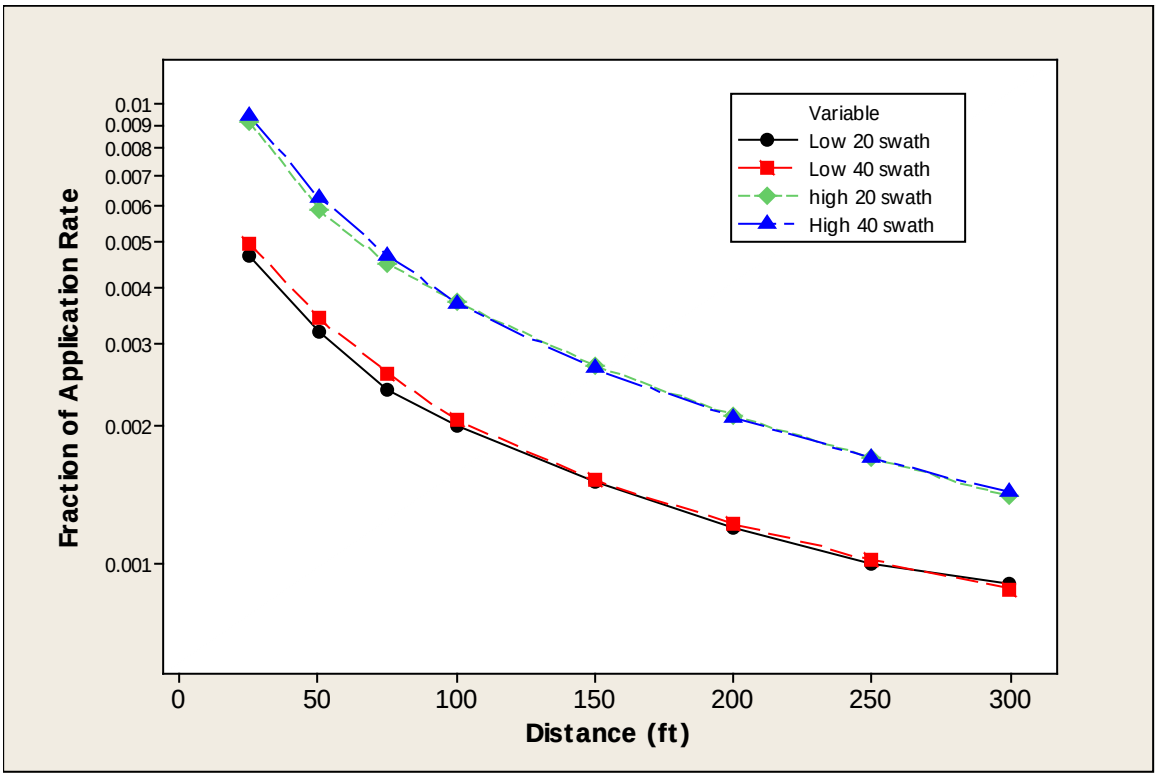
Table 17. Ground boom deposition. High boom and medium/coarse spray quality 20 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.0165	0.3699		25	75	0.0092	0.2062
50	0.0083	0.1861		50	100	0.0059	0.1323
75	0.0057	0.1278		75	125	0.0045	0.1009
100	0.0044	0.0986		100	150	0.0037	0.0829
150	0.0031	0.0695		150	200	0.0027	0.0605
200	0.0023	0.0516		200	250	0.0021	0.0471
250	0.0019	0.0426		250	300	0.0017	0.0381
300	0.0015	0.0336		300	350	0.0014	0.0314

Table 18. Ground boom deposition. High boom and medium/coarse spray quality 40 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
25	0.0166	0.3716		25	75	0.0095	0.2121
50	0.0086	0.1937		50	100	0.0063	0.1408
75	0.0061	0.1375		75	125	0.0047	0.1054
100	0.0046	0.1034		100	150	0.0037	0.0827
150	0.0030	0.0679		150	200	0.0027	0.0596
200	0.0023	0.0524		200	250	0.0021	0.0467
250	0.0019	0.0417		250	300	0.0017	0.0380
300	0.0016	0.0348		300	350	0.0014	0.0321

Figure 5. Ground boom 50 foot width average deposition. Medium/coarse spray quality. Comparison between low boom and high boom. The development procedure for these deposition estimates is described in the text.



Aerial. Deposition estimates for the fixed wing and helicopter scenarios are shown in Tables 19 and 20. A comparison between the AT802A fixed wing aircraft and the Bell 205 helicopter is shown in Figure 6. With the exception of the field edge, the Bell 205 helicopter generally shows less deposition than AT802A fixed wing. The application efficiency is approximately 98% for both the AT802A fixed wing aircraft and the Bell 205 helicopter. This means approximately 98% of the active ingredient released during the application is deposited on-site and 2% is lost by spray drift. The aerial application scenario is 50 swaths, so the application efficiency is higher than a smaller application. For example, a 20 swath application of the same aircraft scenario shows an application efficiency of approximately 95%. However, due to the higher total number of swaths, the downwind horizontal deposition is higher at all distances for the 50 swath application. Therefore, the 50 swath application is the reasonable worst case scenario.

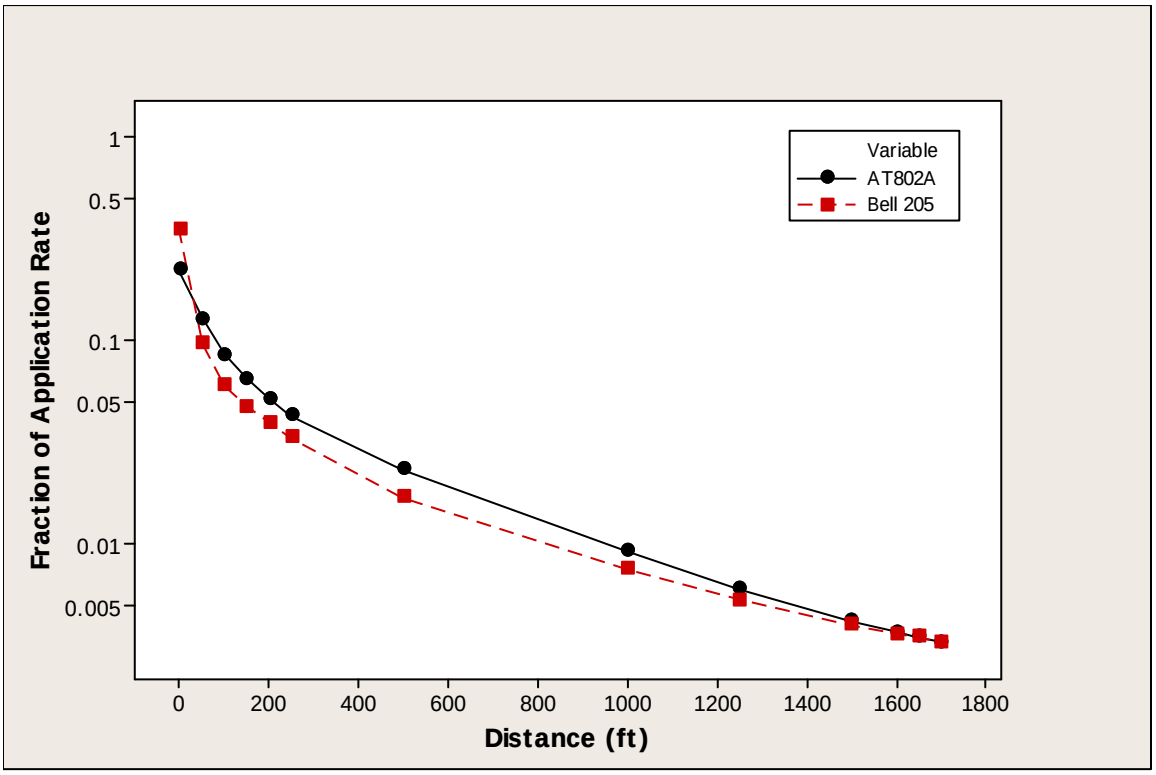
Table 19. Fixed wing aerial application deposition - AT802A medium spray quality 50 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
0	0.3945	8.8435		0	50	0.2259	5.0640
50	0.1644	3.6854		50	100	0.1286	2.8828
100	0.1026	2.3000		100	150	0.0859	1.9256
150	0.0733	1.6432		150	200	0.0652	1.4616
200	0.0577	1.2935		200	250	0.0524	1.1747
250	0.047	1.0536		250	300	0.043	0.9639
500	0.0245	0.5492		500	550	0.0234	0.5246
1000	0.0096	0.2152		1000	1050	0.0092	0.2062
1250	0.0062	0.1390		1250	1300	0.006	0.1345
1500	0.0043	0.0964		1500	1550	0.0042	0.0942
1600	0.0038	0.0852		1600	1650	0.037	0.8294
1650	0.0036	0.0807		1650	1700	0.0035	0.0785
1700	0.0034	0.0762		1700	1750	0.033	0.0740

Table 20. Helicopter aerial application deposition. Bell 205 medium spray quality 50 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

Point Estimates				50 ft Wide Lawn Estimates			
				Location of 50 ft wide Lawn		50 ft Width Average Deposition	
Dist (ft)	Fraction of Rate	2 lb/ac µg/cm^2		Start	End	Fraction of Rate	2 lb/ac µg/cm^2
0	0.8698	19.4983		0	50	0.3584	8.0343
50	0.1427	3.1989		50	100	0.0969	2.1722
100	0.0683	1.5311		100	150	0.0603	1.3517
150	0.0535	1.1993		150	200	0.0479	1.0738
200	0.0434	0.9729		200	250	0.0396	0.8877
250	0.0363	0.8137		250	300	0.0334	0.7487
500	0.018	0.4035		500	550	0.0171	0.3833
1000	0.0077	0.1726		1000	1050	0.0075	0.1681
1250	0.0055	0.1233		1250	1300	0.0053	0.1188
1500	0.0041	0.0919		1500	1550	0.004	0.0897
1600	0.0037	0.0829		1600	1650	0.0036	0.0807
1650	0.0035	0.0785		1650	1700	0.0035	0.0785
1700	0.0034	0.0762		1700	1750	0.0033	0.0740

Figure 6. Aerial application 50 foot width average deposition. Comparison between fixed wing (AT802A) and helicopter (Bell 205). The development procedure for these deposition estimates is described in the text.



Air Concentration Estimates

The AGDISP model produces estimated 1-hr time weighted average (TWA) air concentrations in a vertical plane at user specified downwind distances from the application edge. The air concentration estimates for both the AT802A and Bell 205 were obtained from the same model runs that produced the deposition estimates. Thus, air concentrations were estimated for both the AT802A and Bell 205 aircraft using the 10 mph, 90 deg F, and 20% humidity weather scenario. The vertical plane was set at selected downwind distances, starting with the minimum federal label buffer zone of 10 ft from the application area edge. The 1-hr TWA air concentrations for the vertical plane at the minimum federal buffer zones of 10 ft and at selected heights above ground level are shown in Table 21. Figure 7 shows the change in 1-hr TWA air concentration with height for the vertical planes between 10 ft and 1000 ft downwind of the application edge. At the minimum federal label buffer zone of 10 ft, for the breathing heights of toddlers to adults (1.7 ft and 5 ft, respectively) the Bell 205 helicopter shows the highest 1-hr TWA air

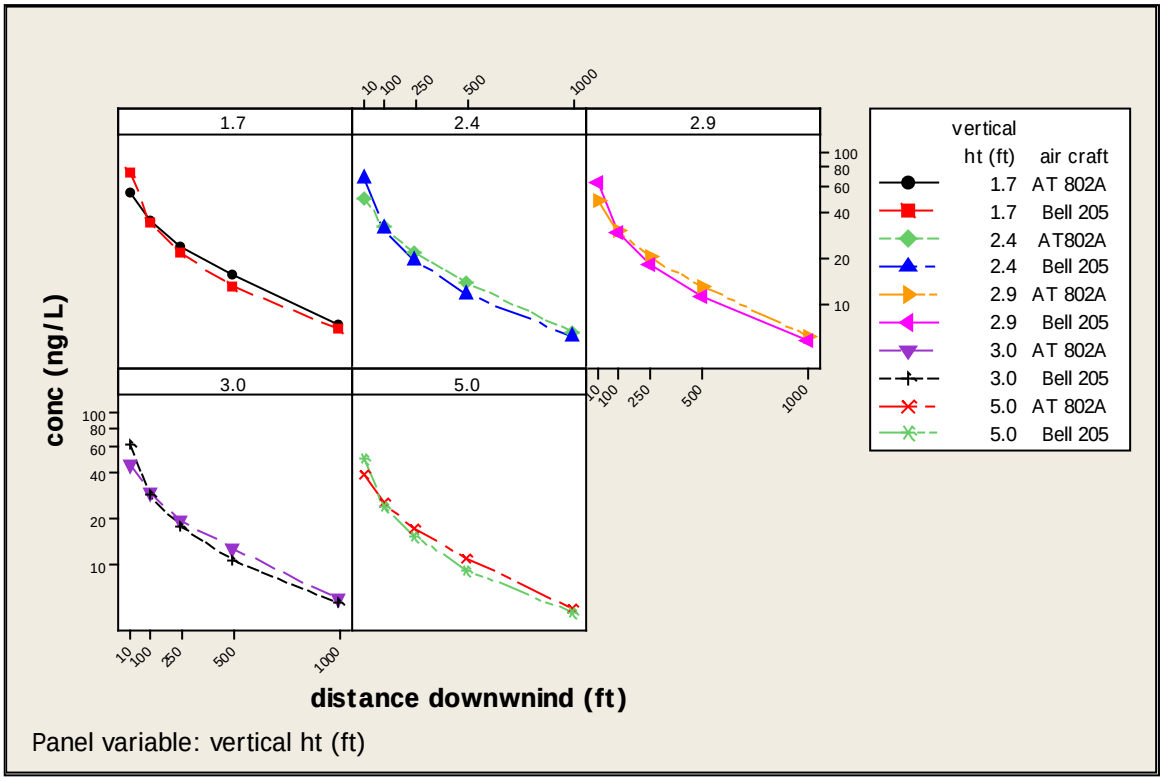
concentration in the vertical plane. As the elevation above ground level increases, however, the 1-hr TWA air concentrations for the AT802A become higher than the Bell 205. The switch occurs at approximately 10 ft above ground level. The AGDISP user manual defines the 1-hr TWA air concentration as: “average concentration of active spray material through a vertical plane at the Transport Distance.” Not all the mass in the cloud passing through the vertical plan at a particular distances will be contained is droplets that are in the inhalable size range. The AGDISP model can output the droplet spectra present and the air concentration vertical plan. Therefore, if desired, a respirable fraction adjustment can be made to the concentration passing through a vertical plan. Complete AGDISP aerial application results are shown in Appendix A.

Table 21. Selected 1-hr time weighted average (TWA) air concentrations (ng/L) in a vertical plane at the federal label minimum buffer zone distance of 10 feet downwind of a 206.6 acres application (20 swaths) with the AT802A fixed wind air craft and a 190.4 acre (20 swaths) application with the Bell 205 helicopter. Development procedures for these air concentration estimates are described in the text.

Height Above Ground		1-Hr TWA Air Concentration (ng/L)	
		Aircraft Model	
Inches	Feet	AT802A Fixed Wing[1]	Bell 205 Helicopter[2]
0	0	n/a[3]	n/a[3]
20	1.7	54.6	72.8
29	2.4	49.6	66.4
35	2.9	47.0	62.5
36	3.0	46.5	61.8
60	5.0	39.9	50.0

[1]Fraction of droplets 10µm or less = 0.0285
[2]Fraction of droplets 10µm or less = 0.0366
[3]The AGDISP model does not estimate air concentrations at ground level.

Figure 7. One hour time weighted air concentrations (ng/L) in a vertical plane at distances between 10 ft and 1000 ft downwind of a 206.6 acres application (20 swaths) with the AT802A fixed wind air craft and a 190.4 acre (20 swaths) application with the Bell 205 helicopter. The development procedure for these air concentration estimates is described in the text.



Comparison of Deposition and Air Concentrations as a function of Finished Spray Volume (GPA) and Application Rate (lb/ac)

The effects of finished spray expressed as gallons per acre (GPA) and the active ingredient (ai) application rate (lb ai/ac) within the same aircraft type and meteorological conditions are examined in this section. There is at least one chlorpyrifos label that requires a minimum of 15 GPA finished spray for certain aerial applications (Cheminova NUFOS 4E USEPA Reg. No. 67760- 28-AA). Based on this label, the two levels of finished spray are modeled: 2GPA (US EPA default) and 15 GPA. Three levels of application rate are also modeled: 1 lb ai/ac, 2 lb ai/ac, and 2.3 lb ai/ac.

The application tank mix scenarios shown in Table 22 were simulated using AGDISP for the fixed wing aircraft AT802A and the rotary wing aircraft Bell205. The 2 GPA tank mix scenarios retain the original aircraft set-ups used in sections above for the chlorpyrifos spray drift analysis.

The 15 GPA scenarios used an aircraft set-up with 60 nozzles on the boom to deliver the higher spray volume. This 60 nozzle spray boom set-up is typical of spray booms used for application of products that require a high GPA finished spray. For example, most propanil labels require a minimum of 10 GPA finished spray for aerial applications with 12-15 GPA recommended in low humidity conditions (e.g. SuperWham!CA EPA Reg. No. 71085-5-ZA and Stam 80 EDF-CA EPA Reg. No. 710085-38-AA). Booms on aircraft performing propanil applications are typically equipped with 50 to 70 nozzles (Rice Research Board, 2001; Rice Research Board, 2002).

The CPF 60 nozzle medium ASAE spray quality aerial boom set-up parameters for the 15 GPA scenario were input into the Aircraft Calibration, Droplet Calculator, and USDA Atomization Model Excel files available for download from the Transland/CP Products Droplet Calculation Tools – Aerial Spray Systems website (http://www.translandllc.com/download/ - Accessed August 8, 2017). The calculators show that several nozzles exist that can deliver a 15 GPA finished spray in the ASAE medium spray quality range using the recommended pressure between 25 and 60 psi. The AGDISP model uses generic inputs of ASAE spray quality, number of nozzles, nozzle spacing, and boom length together with air speed and release height independent of a specific brand of nozzle. Therefore, use of the CP Product calculators is employed simply as a boom system check. It is not required to assume that CP Product nozzles are actually used for this scenario to the exclusion of other nozzle brands.

The base scenario of 2 GPA finished spray volume is the default in both the AGDISP and AgDRIFT models and is the default finished spray volume typically used by USEPA (Dawson et al., 2012). The base scenario application rate is designated as 2 lb ai/ac. Thus, for this analysis the base scenario tank mix is 2 GPA finished spray volume and 2 lb ai/ac. All other tank mix combinations are compared to this base. As stated above, the Cheminova NUFOS 4E insecticide chlorpyrifos formulation (EPA Reg. No. 67760- 28-AA) that has 4 lb ai/gallon (0.5 lb/pint) was used for this simulation because this label requires a minimum of 15 GPA finished spray for some aerial applications. The ai is 45% by volume in this formulation. For all tank mix scenarios the ai is declared non-volatile. The remainder of the product is assumed to be volatile. While other components of the NUFOS 4E formulation may be non-volatile, the exact properties are unknown so the remainder of the formulation is considered volatile. In addition, it is assumed no tank mix additives were used so only the ai is non-volatile.

Table 22. Tank mix calculations for the AGDISP tank mix comparison runs. Cheminova NUFOS 4E insecticide chlorpyrifos formulation (US EPA Registration Number 67760- 28-AA).

2 GPA Finished Spray (16 pints)			
ai[1] rate per acre	formulation volume per acre	Proportion of ai in the tank mix volume	Percent ai in the tank mix volume[2]
1 lb	2 pints	2/16*0.45 = 0.56	6%
2 lb	4 pints	4/16*0.45 = 0.113	12%
2.3 lb	4.6 pints	4.6/16*0.45 = 0.129	13%
15 GPA Finished Spray (120 pints)			
ai rate per acre	formulation volume per acre	Proportion of ai in the tank mix volume	Percent ai in the tank mix volume[3]
1 lb	2 pints	2/120*0.45 = 0.008	0.8%
2 lb	4 pints	4/120*0.45 = 0.015	1.5%
2.3 lb	4.6 pints	4.6/120*0.45 = 0.017	1.7%

[1]Active ingredient
[2]Rounded up to the nearest 1%
[3]Not rounded up to the nearest 1% because the proportion of ai in the tank mix is small.

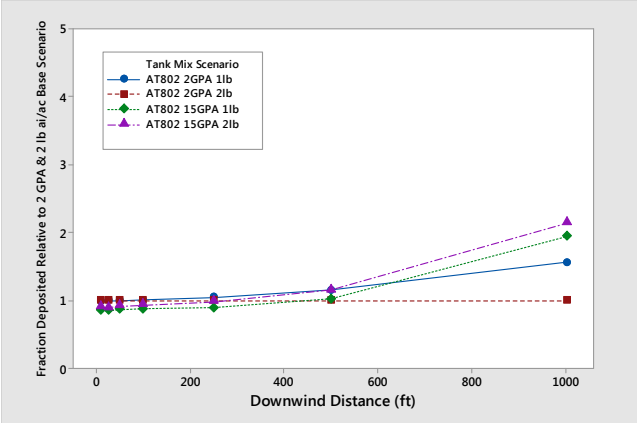
Figure 8 presents results for the AT802A fixed-wing aircraft tank mix scenarios relative to the base tank mix of 2GPA and 2 lb ai/ac (at each distance the scenario result is divided by the result for 2GPA and 2 lb/ac). Comparison of relative changes with scenario and distance can be made between horizontal fraction deposition, horizontal mass deposition, and air concentration in Figure 8 because the results are ratios and the plots are on the same scale. Figure 8a and 8b show the relative deposition of fraction and mass for each scenario, respectively. Figure 8c shows the relative air concentration for each scenario.

Across combinations of finished spray volume and application rates, near field (within about 200 ft of the application edge) the relative horizontal fraction results are reasonably similar (e.g., the fraction of application rate deposition ratio of base tank mix to scenario tank mix is close to 1.0) (Figure 8a). However, the far field results differ between scenarios, ranging from about 1.5 to 2 times the base scenario. Changes in relative fraction deposition are not proportional to differences in tank mix scenarios. Figures 8b and 8c show that changes in relative mass deposition and air concentrations are also not proportional to tank mix scenarios. The 15 gal/ac scenarios show the largest differences regardless of application rate. These results indicate: 1) simple multiplication of a base application rate deposition curve (fraction or mass) to obtain other application rates at the same GPA volume does not produce the same results compared to running the AGDISP model (or AgDRIFT model) separately for each tank mix scenario and 2)

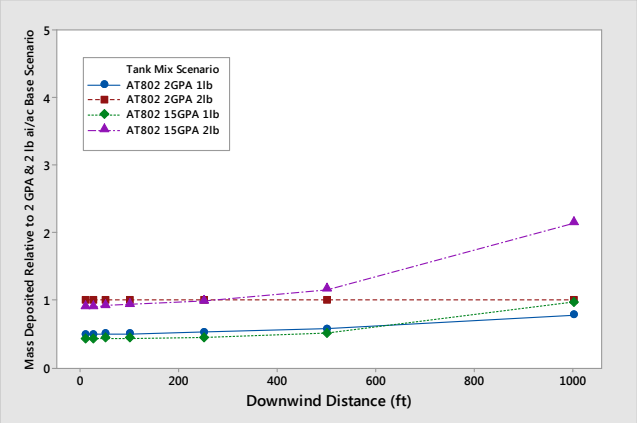
finished spray volume likely affects deposition and air concentration results through differences in the percent of ai in the tank mix. Therefore, these results imply a potential tank mix effect that is not considered if the default inputs alone are used to produce horizontal deposition and air concentration estimates. The higher finished spray volume per acre appears to increase deposition in the far field and increase air concentrations throughout the model domain.

Figure 8. Horizontal deposition (fraction of application rate and mass) and air concentration relative to the base scenario of AT802A aircraft 2GPA finished spray and 2 lb ai/ac application rate (AT802A 2GPA 2lb). Additional scenarios vary combinations of volume of finished spray (GPA) and application rate (lb ai/ac). Results at each distance for each scenario are divided by the result for the base scenario (the vertical axis is dimensionless).

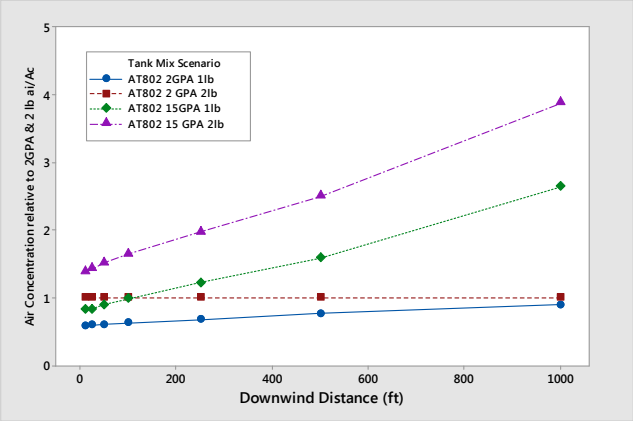
a. Horizontal Fraction Deposition



b. Horizontal Mass Deposition



c. Air Concentration



Comparison with US EPA Results

Both this analysis and the analysis from US EPA used computer simulation models to produce horizontal deposition and air concentration estimates for chlorpyrifos. Inputs for some scenarios modeled were similar. For other scenarios the inputs were quite different.

For orchard airblast and ground boom this analysis used AgDRIFT 2.0.05 because when this analysis was conducted staff did not have access to AgDRIFT 2.1.1 regulatory version. For orchard airblast and ground boom AgDRIFT 2.0.05 yielded identical results to AgDRIFT 2.1.1 public version. After this analysis was finished staff obtained the regulatory version of AgDRIFT 2.1.1. As expected, results for orchard airblast and ground boom were identical between AgDRIFT 2.0.05 and AgDRIFT 2.1.1 regulatory version. That is because the empirical models that produce the orchard airblast and ground boom results have not changed since the versions of AgDRIFT developed following the expert panel review in the mid-1990's. The user manual supplied with AgDRIFT 2.1.1 is the user manual for AgDRIFT 2.0.07 (Teske et al., 2003).

Orchard Airblast. This analysis and US EPA orchard airblast simulations used consistent inputs. The only differences are due to US EPA rounding up to 2 decimal places for the horizontal deposition. US EPA presented only the sparse orchard scenario. This analysis presents sparse orchard, dormant apples, and grape vineyard (non-wrap-around). A side-by-side comparison for sparse orchard and 2 lb ai/ac application rate is shown in Table 23.

Table 23. Comparison of 50th percentile sparse orchard horizontal deposition (lb ai/ac) across a 50ft wide lawn for 20 rows and 2 lb ai/ac application rate as estimated using the AgDRIFT model.

Distance Downwind (ft)	This Analysis	USEPA
0	*[1]	0.57[2]
10	*	0.16
25	0.0886	0.09
50	0.04	0.04
75	0.022	0.02
100	0.0136	0.01
125	0.009	0.01
150	0.0064	0.01
200	0.0036	0.00
250	0.0022	0.00
300	0.0016	0.00

[1]This analysis did not report estimates for empirical model fits between 0 and 25 feet because no field measurements were made within that distance range. The empirical model fit starts at 25 ft downwind of the treated field.
[2]The US EPA field edge horizontal deposition estimates are in error (References: Personal Communication with Charles Peck; US EPA 2014).

Ground Boom. There are no differences between this analysis and USEPA for ground boom simulation inputs. Both used the same scenarios of ASAE Fine to Medium/Coarse droplet spectra for low and high boom applications. However, USEPA reported the 90th percentile estimates. This analysis reported the 50th percentile estimates because the orchard airblast and aerial are both 50th percentile estimates. The use of the 50th percentile estimate puts ground boom on the same estimation basis as orchard airblast and aerial. Table 24 shows a side-by-side comparison of ground boom horizontal deposition (lb ai/ac) across a 50ft wide lawn for 20 swaths and 2 lb ai/ac application rate as estimated using the AgDRIFT model.

Table 24. Comparison of ground boom horizontal deposition (lb ai/ac) across a 50ft wide lawn for 20 swaths and 2 lb ai/ac application rate as estimated using the AgDRIFT model.

Distance Downwind (ft)	This Analysis Low Boom[1] 50th Percentile	USEPA Low Boom 90th Percentile	This Analysis High Boom[2] 50th Percentile	USEPA High Boom 90th Percentile
0	*[3]	0.46[4]	*	0.54[4]
10	*	0.02	*	0.04
25	0.0094	0.02	0.0184	0.03
50	0.0064	0.01	0.0118	0.02
75	0.0048	0.01	0.009	0.02
100	0.0040	0.01	0.0074	0.01
125	0.0034	0.01	0.0062	0.01
150	0.0030	0.01	0.0054	0.01
200	0.0024	0.00	0.0042	0.01
250	0.0020	0.00	0.0034	0.01
300	0.0018	0.00	0.0028	0.01

[1]Low boom height is 20 inches above the target.
[2]High boom is 50 inches above the target.
[3]This analysis did not report estimates for empirical model fits between 0 and 25 feet because no field measurements were made within that distance range. The empirical model fit starts at 25 ft downwind of the treated field.
[4]US EPA field edge deposition estimates are in error (References: Personal Communication with Charles Peck; US EPA 2014).

Aerial. Differences between aerial simulation inputs for this analysis and USEPA produces differences in the horizontal deposition. One difference is that this analysis used AGDISP 8.28 (Teske and Curbishley, 2013) to simulate the aerial application scenarios while USEPA used AgDRIFT 2.1.1 regulatory version. Table 25 follows the format of the AgDRIFT 2.0.05 user's manual and shows the AgDRIFT and AGDISP model inputs (Teske et al., 2002). The format of the AgDRIFT user's manual does not change with model version and the Tier I default parameter are the same between AgDRIFT 2.0.05 and AgDRIFT 2.1.1. The AgDRIFT Tier I

default inputs shown in Table 25 were not changed by USEPA from those defaults for the AgDRIFT Tier II model runs.

Table 25. Details of Aerial Application inputs for AGDISP and AgDRIFT this analysis and USEPA, respectively.

	This Analysis AGDISP	USEPA AgDRIFT
Aircraft Model	AT802A	AT401
Weight	11160 lbs	6000 lbs
Wing Semispan	29 ft	24.5 ft
Flight Speed	144.99 mph	119.99 mph
Release Height	10 ft	10 ft
Number of Nozzles	39	42
Vertical Offset	-0.6601 ft	-1.51 ft
Horizontal Offset	-0.5 ft	-0.83 ft
Boom Span	76.3%	76.32%
Spacing (even)	14 inches	11 inches
ASABE[1] Droplet Spectra Classification	Medium	Tier I Fine to Medium Tier II Medium
Wind Speed at 2 m	10 mph	10 mph
Wind Direction	Perpendicular to Flight Path	Perpendicular to Flight Path
Surface Roughness	0.12 ft (low crops)	0.0246 ft (bare soil)
Stability	Overcast (Neutral)	Overcast (Neutral)
Relative Humidity	20%	50%
Temperature	90 deg F	86 deg F
Specific Gravity	1.0	1.0
Spray Volume Rate	2 gal/ac	2 gal/ac
Application Rate	2 lb/ac[2]	2 lb/ac
Nonvolatile Rate	2 lb/ac	3 lb/ac[3]
Active Solution % of Tank Mix	12%	12%
Additive Solution % of Tank Mix	0%	5%
Nonvolatile Active	12%	12%
Volatile Fraction	0.88	.83
Nonvolatile Fraction	0.12	.17
Swath Width	60 ft	60 ft
Swath Displacement	37%	37%
Number of Flight Lines	50	20

[1]American Society of Agricultural and Biological Engineers. Formerly American Society of Agricultural Engineers (ASAE). The organization change names in 2005.

[2]Application rates of 1, 2, 2.3, 4, and 6 lb/ac were simulated both 2 gal/ac and 15 gal/ac spray volume.

[3]US EPA indicates in D3399483. AppendixF.CPOSDrift.xlsx "…DAS Error Correction Comments/Meetings" for this tank mix but there is no accompanying documents to explain the "correction." Not all chlorpyrifos products are Dow products so this analysis does not include the 1 lb/ac of non-ai nonvolatile material in the tank mix. https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0850-0107

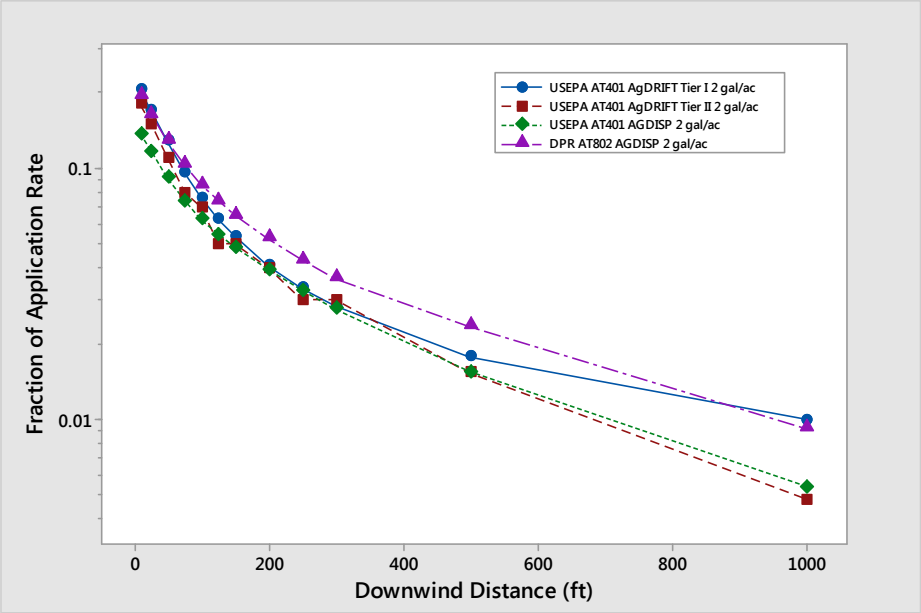
Deposition estimates for 2 lb ai/ac application rate are compared in Table 26 and shown in Figure 9. For this comparison, USEPA AgDRIFT estimates were extended to 1000 ft downwind to match the AGDISP estimates. In addition, the USEPA AgDRIFT inputs were used in AGDISP to provide a comparison of AgDRIFT and AGDISP horizontal deposition estimate for the AT401 aircraft. The AgDRIFT 2.1.1 aerial algorithm does not include an evaporation time-step refinement that was incorporated into AGDISP 8.28 to improve mass accountancy (H. Thistle, pers. comm., 2014). This results in the AgDRIFT horizontal deposition being higher than AGDISP for the same scenario (AT401 aircraft/20 swaths) due to the lack of the refined evaporation time-step. This effect is apparent in Figure 9 because the AGDISP results using the USEPA AT401 inputs show lower horizontal deposition relative to the AgDRIFT AT401horizontal deposition results. This analysis used AGDISP. However, the horizontal deposition estimates reported in this analysis are higher relative to USEPA horizontal deposition estimates for several reasons: 1) the AT802A was selected as the California aircraft based on common use in California and higher horizontal deposition estimates, 2) this analysis used 50 swathes (USEPA used 20 swaths) to reflect the largest application sizes in California, 3) the meteorological conditions used in this analysis are California specific, and 4) the tank mix fractions used in this analysis are California specific.

Table 26. Comparison of aerial horizontal deposition (fraction of application rate) across a 50ft wide lawn for 2 lb ai/ac application rate as estimated using the AgDRIFT and AGDISP models.

Downwind Distance (ft)	USEPA AgDRIFT 2 gal/ac 20 swath AT401 Tier I	USEPA AgDRIFT 2 gal/ac 20 swath AT401 Tier II	USEPA Inputs AGDISP 2 gal/ac 20 swath AT401	This Analysis AGDISP 2 gal/ac 50 swath AT802A
10	0.20	0.1840	0.1374	0.1929
25	0.17	0.1475	0.1170	0.1640
50	0.13	0.1125	0.0914	0.1286
75	0.10	0.0854	0.0742	0.1034
100	0.08	0.0682	0.0627	0.0859
125	0.06	0.0570	0.0546	0.0739
150	0.05	0.0496	0.0483	0.0652
200	0.04	0.0394	0.0394	0.0524
250	0.03	0.0324	0.0327	0.0430
300	0.03	0.0271	0.0275	0.0365
500	0.02	0.0154	0.0155	0.0234
1000	*[1]	0.0048	0.0054	0.0092

[1]AgDRIFT Tier I does not estimate to 1000 ft.

Figure 9. Aerial application horizontal deposition estimates expressed as fraction of 2 lb ai/ac application rate as modeled by 4 different AgDRIFT and AGDISP scenarios.



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Appendix A – AGDISP Full Results for Aerial Application Scenarios

AT802A
2 GPA
1 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.1922	31.8	23.4	0.0341
25	0.1639	29.2	21.8	0.0357
50	0.1290	26.4	19.4	0.0376
100	0.0869	22.0	16.3	0.0406
250	0.0453	16.1	11.8	0.0471
500	0.0270	11.7	8.5	0.0570
1000	0.0144	6.5	4.7	0.0852
1320	0.0094	4.6	3.3	0.1072
2608	0.0017	1.6	1.2	0.2290

Bell205
2 GPA
1 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.2454	40.9	28.8	0.0440
25	0.1553	33.6	24.0	0.0472
50	0.0951	27.4	19.7	0.0510
100	0.0578	21.9	15.8	0.0558
250	0.0369	15.3	11.1	0.0662
500	0.0219	10.2	7.4	0.0831
1000	0.0107	5.8	4.2	0.1178
1320	0.0075	4.5	3.2	0.1410
2608	0.0012	2.0	1.5	0.2500

AT802A
2 GPA
2 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.1929	54.6	39.9	0.0285
25	0.1640	49.3	36.7	0.0300
50	0.1286	43.7	32.0	0.0321
100	0.0859	35.0	25.9	0.0355
250	0.0430	23.7	17.4	0.0440
500	0.0234	15.3	11.1	0.0589
1000	0.0092	7.2	5.2	0.0999
1320	0.0054	4.9	3.6	0.1300
2608	0.0010	1.6	1.2	0.2800

Bell205

2 GPA
2 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.2471	72.8	50.0	0.0366
25	0.1574	58.0	40.4	0.0400
50	0.0969	45.8	32.2	0.0445
100	0.0603	34.5	24.6	0.0500
250	0.0334	21.5	15.4	0.0640
500	0.0171	13.0	9.3	0.0867
1000	0.0075	6.8	4.9	0.1329
1320	0.0048	4.99	3.61	0.1600
2608	0.0008	2.19	1.59	0.2887

AT802A
2 GPA
2.3 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.1929	58.3	42.8	0.0283
25	0.1639	52.6	39.4	0.0302
50	0.1284	46.4	34.1	0.0324
100	0.0856	37.1	27.5	0.0360
250	0.0428	25.0	18.3	0.0451
500	0.0227	15.9	11.5	0.0605
1000	0.0088	7.5	5.4	0.1026
1320	0.0050	5.1	3.7	0.1333
2608	0.0011	1.7	1.2	0.2951

Bell205
2 GPA
2.3 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.2472	77.1	53.8	0.0376
25	0.1575	61.1	43.5	0.0413
50	0.0970	48.2	34.5	0.0458
100	0.0605	36.2	26.0	0.0521
250	0.0328	22.2	16.0	0.0675
500	0.0165	13.3	9.6	0.0915
1000	0.0071	6.9	5.0	0.1405
1320	0.0045	5.0	3.7	0.1753
2608	0.0009	2.3	1.6	0.3127

AT802A
15 GPA
1 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.1671	44.3	32.3	0.0737
25	0.1409	41.3	30.6	0.0749
50	0.1127	39.1	28.7	0.0765
100	0.0754	34.8	25.6	0.0788
250	0.0387	28.9	21.2	0.0826
500	0.0240	24.3	17.7	0.0863
1000	0.0179	19.0	13.8	0.0944
1320	0.0162	16.4	11.9	0.1011
2608	0.0048	9.0	6.5	0.1468

Bell205
15 GPA
1 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.2281	68.5	48.7	0.0920
25	0.1403	59.2	42.6	0.0958
50	0.0814	51.7	37.3	0.0994
100	0.0472	44.8	32.5	0.1026
250	0.0328	36.7	26.6	0.1102
500	0.0246	28.8	20.9	0.1200
1000	0.0161	20.2	14.7	0.1410
1320	0.0129	15.0	10.8	0.1558
2608	0.0021	8.0	6.4	0.2140

AT802A
15 GPA
2 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.1738	75.8	55.3	0.0565
25	0.1472	70.3	52.2	0.0577
50	0.1186	66.0	48.4	0.0590
100	0.0808	57.9	42.6	0.0615
250	0.0425	46.8	34.2	0.0677
500	0.0271	38.1	27.8	0.0710
1000	0.0197	27.9	20.2	0.0835
1320	0.0171	22.7	16.5	0.0936
2608	0.0041	10.3	7.5	0.1606

Bell205
15 GPA
2 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.2343	96.7	68.6	0.0708
25	0.1461	82.8	59.6	0.0741
50	0.0870	71.5	51.6	0.0776
100	0.0515	61.2	44.3	0.0814
250	0.0360	48.8	35.3	0.0889
500	0.0256	37.3	27.0	0.1008
1000	0.0155	25.2	18.3	0.1240
1320	0.0118	20.7	15.0	0.1390
2608	0.0021	11.5	8.3	0.2040

AT802A
15 GPA
2.3 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.1745	84.1	61.4	0.0574
25	0.1480	77.9	57.9	0.0587
50	0.1194	73.0	53.6	0.0602
100	0.0813	63.7	46.9	0.0629
250	0.0429	51.3	37.5	0.0676
500	0.0273	41.5	30.3	0.0735
1000	0.0198	29.9	21.7	0.0875
1320	0.0167	24.1	17.5	0.1001
2608	0.0041	10.6	7.7	0.1740

Bell205
15 GPA
2.3 lb ai/ac

distance downwind (ft)	horizontal deposition (fraction)	Air concentration (ng/L) at 1.7 ft	Air concentration (ng/L) at 5.0 ft	fraction <=10um
10	0.2355	107.4	76.2	0.0732
25	0.1472	91.7	65.9	0.0759
50	0.0879	78.9	56.9	0.0804
100	0.0522	67.1	48.5	0.0851
250	0.0362	53.2	38.5	0.0926
500	0.0254	40.2	29.1	0.1058
1000	0.0154	26.9	19.5	0.1313
1320	0.0117	22.0	15.9	0.1481
2608	0.0021	12.7	9.2	0.1769

APPENDIX 3.

ASCIX INPUT FILE (M-FILE) FOR USE IN GENERATING THE INHALATION POINT-OF-DEPARTURE

Appendix 3: asclX Input file (m-file) for use in generating the inhalation point-of-departure

```
Human_Parameters_MRP                    % Sets up all human parameters
preg_female_parameters                  % US EPA used female
BWSW=1;                                 % Sets model to run based on body weight or age
BWST=11;                                % Body weight, children 1-2 years old
VVOL=0.025;                             % Child urinary volume approx.
AGE0=1.5;
CONCMGM=2.85;
CINT=2;
TSTOP=504;                              % 504 hours = 21 days
%exposure timing commands
D3IN=7;  % DAYS/WEEK  for acute, set =1, for every day = 7
P2IN=1;  % HRS/DAY for acute, set =1, for 1 hr daily set =1, to match EPA Table 1 =2 hr/day
W2IN=21; % Days of repeated exposure

prepare @clear@all
start @NoCallback

simall = [_time _rbcce _urinetcpy _cv*350.6 _cvo*334.5 _blauc*350.6 _blauco*334.5];%conc unit = ug/L
URINETCPY  %ug/L
min(_rbcce)
!! plo rbcce
!! plo urinetcpy

save simall @file='Inhalation_Child_SS_DPR' @format=ascii
```