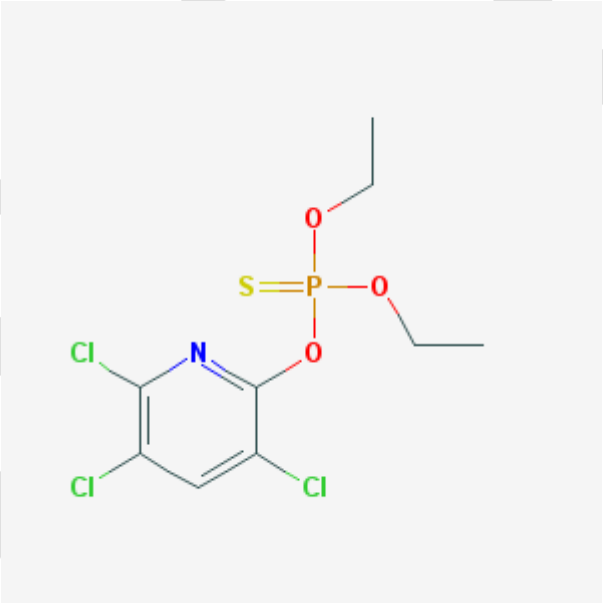
CHLORPYRIFOS

RISK CHARACTERIZATION DOCUMENT

Spray Drift, Dietary and Aggregate Exposures to Residential Bystanders



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AADD	Annual Average Daily Dosage
AC	Adenylcyclase
ACh	Acetylcholine
AChE	Acetylcholinesterase
ADD	Absorbed Daily Dosage
AEA	Anandamide
2-AG	2-arachidonoylglycerol
a.i.	Active Ingredient
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower limit (95th percentile)
BuChE	Butyryl/plasma/pseudo-ChE or B-esterase
CalEPA	California Environmental Protection Agency
Cal PIQ	California Pesticide Illness Query
cAMP	Cyclic AMP
CDPR	California Department of Pesticide Regulation
CES	Carboxyesterase
CNS	Central Nervous System
CPF	Chlorpyrifos
CPF-oxon	Chlorpyrifos oxon
DA	Dopamine
DAP	Dialkylphosphate
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 (1996)
GABA	ɣ-aminobutyric acid
GD	Gestation Day
GnRH	Gonadotrophin Releasing Hormone
HHAB	Human Health Assessment Branch
HDT	Highest Dose Tested
5HT	Serotonin
HTS	High Throughput Screening
IARC	International Agency for Research on Cancer
i.p.	Intraperitoneal
IRED	Interim Reregistration Elegibility Decision
LADD	Lifetime Average Daily Dose
LD	Lactation Day
LDT	Lowest Dose Tested
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Obs Effect Level
LOD/LOQ	Limit of Detection/Limit of Quantification
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 Defence 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	Urinary metabolite 3,5,6-trichloro-2-pyridinol
ToxCast	U.S.EPA Toxicology Forcaster
ToxPi	Toxicological Priority Index
UF	Uncertainty Factor
U.S.EPA	U.S. Environmental Protection Agency

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. 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).

CPF was given a "High" priority status by the California Department of Pesticide Regulation (CDPR), due to concerns regarding (1) potential neurodevelopmental/ neurobehavioral effects from exposures during vulnerable developmental windows in fetuses, infants and children, (2) genotoxicty and reproductive toxicity in rats (3) probable human exposure due to spray drift, (4) possible infantexposure 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 CDPR's process of 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).

This Risk Characterization Document addresses potential human effects arising from exposure to CPF from food, drinking water, air and skin 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 manifactured by Dow AgroSciences 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 2001, all residential uses and uses in schools and parks were prohibited and many agricultural uses were restricted in the U.S. Currently, CPF is only registered to control insects on agricultural crops and for public health to control of mosquitos in the United States. California is the only state that regulates CPF as restricted use material (http://www.cdpr.ca.gov/docs/legbills/rulepkgs/14-002/final_text.pdf).

USES IN CALIFORNIA

To date, there are 49 actively registered product labels in California including 4 master labels. Among those, 21 products have labeling language that specifiesaerial and (or) ground application methods. The total yearly use of CPF ranged from 1.10 (low in 2012) to 1.46 (high in 2013) million pounds to 0.9 to 1.3 million acres with the average of 1 lb/acre.). While these quantities were at their highest in the most recent year reported (2013), they nonetheless fluctuate from year to year. Almonds received the highest poundage of CPF (range: 192,482 in 2012 to 448,673 lb in 2013) compared to other crops.

ILLNESS REPORTS

In California from 2003 to 2012, 225 cases involving CPF were reported. Of these, 104 involved CPF as the sole active ingredient used. Three cases involved occupational users, where mixer/loaders or applicators were exposed to direct spray/drift because they were not wearing the appropriate personal protective equipment (PPE). Other cases were residential exposures to pesticide mixtures containing CPF,

with 1 case (1 episode) involving a child ingesting a pesticide mixture that was in an unmarked drinking container and 5 cases (1 episode) involving a family of 5 becoming ill after an illegal application in their mobile home.

TOXICOLOGY PROFILE

The neurotransmitter acetylcholine (ACh) is hydrolyzed by cholinesterase enzymes (ChE), which are serine hydrolases. AChE hydrolyzes ACh at synaptic clefts in the central nervous system (CNS), at the neuromuscular or neuro-glandular junctions in the peripheral nervous system (PNS) 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 (PNS) or neural stimulation in the central nervous system (CNS). Butyrylcholinesterases (BuChE), which represent the majority of the ACh-hydrolyzing activity in human plasma, are also inhibited by CPF, though the toxicologic consequences 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 (CYP, CYP1A2, 2B6, 2C19, 3A4, 3A5, and 3A7). Oxidative desulfuration results in CPF-oxon. Dearylation of CPF and CPF-oxon by CYP produces TCP 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 3,5,6-trichloro-2-pyridinol (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 U.S. EPA as a moderate oral toxicant (Category II). The acute oral LD50 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. Theermal LD_{50} in rats is 202 mg/kg/day. The 4-hour inhalation LC_{50} in rats is >2 mg/L. CPF is a Category IV skin and eye irritant (slight conjunctival and dermal irritation). Human deaths are reported due to accidental exposure or intentional ingestion. CPF doses >300 mg/kg in humans resulted in unconsciousness, convulsions, cyanosis and uncontrolled urination.

The main targets of CPF toxicity after short-term oral exposure are the nervous system and developing organisms. Cholinergic syndromes from overstimulation of the muscarinic and nicotinc ACh receptors include hypersalivation, respiratory distress, miosis, muscular twitches, tremors, ataxia, diarrhea and vomiting. Other effects are hematological and liver enzyme changes, chromodachtyorhea, 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 (NOEL) for CPF are typically based on RBC or brain AChE inhibition, for which robust data in animals and humans are available. With respect to RBC AChE inhibition, young animals are generally more sensitive than adults, and female animals are more sensitive than males. A Benchmark Dose (BMD) analysis, performed by the U.S. EPA (2011), calculated a BMDL (lower bound of BMD) of 0.36 mg/kg/day 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, the U.S. EPA used the Physiologically-Based Pharmacokinetic-Pharmacodynamic (PBPK-PD) model to estimate the critical NOELs or toxicological point of departures (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) exposures (Summary Table 1). The acute PoDs for children and women of childbearing age were 0.5-0.6 mg/kg/day and the steady state PoDs were 0.08-0.1 mg/kg/day.

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, the following effects were reported: increased adrenal gland, brain and heart weight in rats, increased liver weight and hepatocyte vacuolation in dogs and mice and ocular opacity and hairloss in mice. In 2011, U.S. EPA established a chronic BMDL of 0.09 mg/kg/day based on 10% RBC ChE inhibition in PND 11 male rats after 11 days of oral exposures.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

The available 2- and 3-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 malformations were observed in the presence of maternal toxicity.

DEVELOPMENTAL NEUROTOXICITY

CPF causes developmental neurotoxicity in rats and mice at doses that elicit minimal or no fetal brain AChE inhibition. Three major prospective cohort studies studies in humans evaluated pre- and post-natal pesticide exposure in mother-infant pairs and birth and developmental outcomes in neonates, infants and children. The study from the Columbia University in New York City 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 TCP (a metabolite of CPF and CPF methyl) and non-specific OP metabolites in maternal urine. Collectivly, the results from these studies showed associations of indoor and outdoor exposure to CPF during pregnancy with adverse neurodevelopmental outcomes in children through age 11 years, including delays in cognitive and motor functions, problems with attention, tremors and respiratory symptoms.

GENOTOXICITY

CPF is negative for gene mutation (*Salmonella typhimurium*, *Eschericia 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.

IMMUNOTOXICITY

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

TOXICITY FORECASTER (ToxCast) PROFILES

The *in vitro* ToxCast high-throughput screening assays (HTS), including *in vivo* zebrafish (ZF) assays were examined for indications of pathway disruptions that could lead to toxic effects.. While CPF was not active for human and rat AChE in the ToxCast assays, its oxon metabolite was, indicating that metabolic activation is probably required for inhibitory activity in these assays. For CPF, positive ToxCast assays included cell adhesion, cell cycle and cell morphology assays. However, it is unclear if these pathways impacted by CPF are potential noncholinergic mechanisms responsible for the observed CPF neurodevelopmental toxicity *in vivo*.

The zebrafish model showed embryos with chorion intact could metabolize CPF to a toxic metabolite. CPF induced embryonic malformations and neurobehavioral effects (AChE inhibition, average choice accuracy, decreased spatial discrimination, increases in average latency response, decreased swimming activity, decreases in habituation of swimming activity).

Persistent effects from hatching to adults included a decline in ZF brain dopamine and norepinephrine levels, decreased habituation to startle, increased startle response, decreased escape diving response, increased swimming activity and lower learning rate. CPF affected anxiety-related behaviors in ZF (decreased swim speed and thigmotaxis [edge preference/anxiety]). The active concentration of CPF on AChE inhibition in ZF was s 0.1 μM. At concentrations not inhibiting AChE (i.e., 0.01 μM), CPF caused significant increase in abnormal behavioral (increased "fish at rest", decreased swim speed, decrease in fish with a preference for being on the side or on the edge of their swim lane). At 10-fold lower CPF concentrations than those inhibiting AChE, ZF behaviors were affected during embryonic development.

RISK ASSESSMENT

The health risk assessment of CPF (CPF) was carried out for 4 sentinel subgroups of the general population: infants (<1 years old), children 1-2 years, children 6-12 years, and women of childbearing age (13-49 years).

HAZARD IDENTIFICATION

In this risk assessment, the critical acute and subchronic endpoints or toxicological PoDs for CPF are based on inhibition of the RBC AChE activity. HHAB used the U.S. EPA (2014) estimated PoDs derived from the PBPK-PD model. The PoDs are 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. 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 shows the critical NOELs and endpoints selected by HHAB for evaluating oral, dermal and inhalation exposure from diet and spray drift.

Summary Table 1. Critical NOELs for CPF and CPF-Oxon

Exposure Route[a]	PBPK-PD PoDs[a]									
	Infants < 1 yr old		Children 1-2 yrs		Child 6-12 yrs old		Youths 13-19 yrs old		Females 13-49 yrs old	
	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]
Dietary (Drinking Water or Food-only) Exposure										
Drinking Water										
CPF-oxon ppb	1,183	217	3,004	548	7,700	1,358	4,988	878	5,285	932
CPF-oxon mg/kg/day[c]	0.170		0.159		0.143		0.127		0.129	
Food CPF mg/kg/day	0.600	0.103	0.581	0.099	0.530	0.090	0.475	0.080	0.467	0.078
Spray Drift Exposure to Bystanders										
Oral (mg/kg)	--	--	--	0.099	--	--	--	--	--	--
Dermal (mg/kg/day)	--	--	--	134.25	--	--	--	--	--	23.60
Inhalation (mg/m^3)	--	--	--	2.37	--	--	--	--	--	6.15

a- PoDs are PBPK-PD-estimated human equivalent doses based on 10% inhibition of the RBC AChE activity after an acute (single day, 24 hr) or steady-state (21-day) exposure of CPF in humans (U.S. EPA, 2014).

a- Parent compound CPF for all estimates, except for drinking water where CPF-oxon exposure is estimated.

b- SS = Steady-state: HHAB used SS oral (non-dietary), dermal and inhalation PoDs to estimatethe risk form spray drift and aggregate exposures, since crop treatment occurred at 10 day intervals and plasma ChE and RBC AChE inhibition takes approximately 26 -days to reverse to normal values (Nolan et al. 1984).

CPF, chlorpyriofos

CPF-oxon, chlorpyriofos-oxon

c- Acute PoDs for CPF-oxon in ppb (ug/L) were converted into internal doses (mg/kg/day) using default drinking water consumption and body weight values (see Table 20 in RCD).

EXPOSURE ASSESSMENT

Spray Drift Residue Exposure Estimates

The exposure associated with spray drift near the application site was evaluated for two of the sentinel population subgroups: children 1-2 years, and women of childbearing age (13-49 years). Females 13-49 years old were of interest because of their potential increase in susceptibility to CPF toxicity during pregnancy. The U.S. EPA residential SOP identifies activity patterns associated with children in the 1-2 yrs as resulting in the highest exposure potential to CPF residue on: 1) turf; 2) contaminated lawn via direct dermal contact and (or) mouthing such as hand-to-mouth, object-to-mouth, and 3) incidental soil ingestion. The SOP assumed that the duration of exposure for females 13-49 and children 1-2 years near the application sites would be 1.5 hours.

Aerial Applications

Spray 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 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 rotor-wing (Bell 205 helicopters). Increases in CPF application rate resulted in a corresponding increase in the drift desposition exposure estimates (regardless of exposure routes) 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 drift deposition and inhalation exposure estimates, an additional AGricultural DISPersion (AGDISP) simulation was performed. For a given application rate, the drift exposure estimates appear to be insensitive to the change in spray volumes. By contrast, the estimated 1 hour time-weighted average air concentrations increase with the spray volume.

Ground-Based Applications

The drift deposition exposure estimates (in µg/kg/day) of CPF were evaluated for the same two population subgroups at four maximum allowable application rates with two ground-based application methods: groundboom and airblast. For groundboom, drift deposition estimates were derived using two swath percentiles: 50^{th} and 90^{th}. Drift deposition exposure estimates of CPF for children 1-2 years after groundboom or airblast application showed that exposure increases with application rates of CPF. The higher drift exposure estimate of the high-boom compared with the low-boom is consistent with the difference in canopy interception between the two elevations. Also, the higher drift exposure estimates with orchard airblast compared to groundboom are consistent with the lower spray interception from low canopy density found in dormant apple and sparse orchards compared to normal orchards.

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 (2009-2013), the top ten agricultural uses in the state were almond, citrus, alfalfa, walnut, cotton, grapes, corn, broccoli, sugar beet, and peach/nectarine.

In 2014, the U.S. 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, HHAB determined that it is not necessary to conduct an independent dietary exposure assessment at this time and utilized the 2014 U.S. EPA food-only exposure estimates to evaluate the risk from CPF exposure from food. However, HHAB conducted its own 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-days, steady-state) food-only exposures were calculated for four sentinel subpopulations identified in the U.S. EPA risk assessment: infants (< 1 year old), children 1-2 years, children 6-12 years, and females 13-49 years. Children 1-2 years old were identified as the highest exposed population subgroup: at the 99.9^{th} percentile acute exposure was 0.000423 mg/kg/day and steady-state exposure was 0.000242 mg/kg/day.

Drinking Water Exposure Assessment

CPF is rapidly oxidized to the oxon during the chlorination process. In this assessment, HHAB assumed that 100% of CPF is converted to CPF-oxon during water treatment. HHAB estimated drinking water probabilistic exposures using (*1*) Pesticide Data Program (PDP) drinking water residue data for CPF or (*2*) CPF residue data from the CDPR 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 resides 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 and 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 LOD for CPO. The 99.9th percentile exposure for all infants, the most highly exposed subpopulation, was 0.000108 mg/kg.

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

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

For surface water, between 2005 and 2014 a total of 7,154 samples were analyzed for CPF.The range of detected residues was 0.000572 to 3.7 ppb. For ground water, 2,055 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 yr, were 0.000419 mg/kg (surface water) and 0.000222 mg/kg (ground water).

RISK CHARACTERIZATION

The critical NOELs for characterizing the risk from exposure to CPF were PBPK-PD-estimated human equivalent doses. Risks were calculated as margin of exposure (MOE), a quotient of the NOEL and the human exposure level. A target MOE of 100 was 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 and route-specific NOELs are used, a combined MOE for all routes can be calculated.

Non-Occupational Spray-Drift Bystander MOEs

For spray drift, the duration of exposure for females 13-49 years and children 1-2 years near the application site were assumed to be 1.5 hours. This amounts to an acute duration (less than 1 day). However, 21-day (steady state) PBPK-PD PoDs were used for calculating the MOEs for spray drift exposure. The reasons for employing steady state PoDs instead of acute PoDs were: (1) the product application frequencies are

specified as ≥10 days, and thus exposure to CPF due to off-site movement could be considered as a series of short-term (<1 day) exposures (2) For spray drift, the duration of exposure for females 13-49 yrs and children 1-2 yrs near the application site was assumed to be 1.5 hours. This amounts to an acute duration (less than 1 day). However, 21-day (steady state) PBPK-PD PoDs were used for calculating the MOEs for spray drift exposure. The reasons for employing steady state PoDs instead of acute PoDs were: (1) the product application frequencies are specified as ≥10 days, and thus exposure to CPF due to off-site movement could be considered as a series of short-term (<1 day) exposures (2) studies in humans show that CPF inhibits RBC AChE activity after a single dose, but the enzyme activity does not recover to 100% even after 10 days, suggesting that under the product application frequencies, the inhibitory effect of CPF could be cumulative.

Acute MOEs were estimated for females 13-49 years and children 1-2 years old that were exposed at 10-1000 feet from CPF treated fields. Different 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 (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 (1.5 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 routes. The current buffer zone for CPF is 25 feet.

Females 13-49 years: The MOEs for dermal and inhalation 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; groundboom and airblast at the application rates of 1, 2, 4, or 6 lb a.i./acre.

Children 1-2 years: 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 at 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.

Dietary (food only) Exposure MOEs

At the 99.9th percentile, the acute dietary MOEs from exposure to CPF residues in food ranged from 1,374 to 3,127 for the four sentinel population subgroups. At the 99.9th percentile, the steady state MOEs for these subpopulations ranged from 409 to 1,040. 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 sentinel populations were based on drinking water residues from PDP or from the CDPR surface and ground water residues. At the 99.9th percentile, the MOEs were highest for PDP (1571-3970) and lowest for the CDPR surface water (405 – 1,299). All MOEs for acute water-only exposure were greater than the target of 100.

Aggregate Exposure MOEs

Aggregate Exposure- Combined MOEs (Dietary [food only], Drinking Water [PDP or CDPR Surface Water], Spray-Drift)

For aggregate exposures, it was assumed that a child 1-2 years old will be exposed at 10-1000 feet from the CPF application site potentially through inhalation, skin contact with residues (drift deposition), ingestion of residues by object-to-mouth + hand-to-mouth + incidental soil ingestion (oral exposure), and consumption of food and drinking water (oral, upper bound of exposure [99th percentile]). An aggregate MOE approach was used because of different exposure routes and durations, and route-specific NOELs.

The PoD values used for the risk characterization of aggregate exposures to children 1-2 years are shown in Summary Table 1. For the combined deposition, the risk was calculated using the 21-day steady state dermal, inhalation and oral PoDs for CPF and the acute (1.5 hours) dermal, inhalation, and non-dietary oral exposures. The acute dietary risk from food-only or drinking water probabilistic 99th percentile exposures was calculated using the acute oral PoD for CPF and the acute oral PoD for CPF-oxon, respectively. The drinking water exposures were based on residues from PDP or the CDPR EMON surface water program.

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

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

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 air exposure had a substantial contribution (up to 95%) to the aggregate exposure. Consequently, the combined MOEs were significantly reduced when air exposure was added to the dermal, non-dietary oral and dietary exposures. Therefore, inhalation of air near the application site was the critical exposure driving the aggregate MOEs below the target value of 100 for children 1-2 years (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*) The current PBPK-PD model lacks critical data on physiological changes during pregnancy and AChE genetic variability. Based on only a few human *in vitro* samples the model generates metabolism-related parameters that are meant to be applied to the general population.

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

The main uncertainties in the exposure assessment were:

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

(*ii*) There were no air concentration estimates available for groundboom and airblast applications.

The main uncertainties in the dietary exposure assessment were:

(*i*) Illegal residues measured in foods were not included in the dietary exposure assessment. PDP frequently detected CPF residues on crops that lack tolerances. From 2012 to 2014, the CDPR Residue Monitoring Program detected illegal CPF residues on a high number of mostly imported fresh produce samples 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

HHAB does not evaluate illegal residues on agricultural commodities in its dietary exposure assessments. Such residues come under the purview of CDPR's Enforcement Branch, which has the authority to remove affectedproduce from channels of trade. Nevertheless, the high frequency of CPF detections heightens the risk of additional exposures not considered in the dietary assessment.

(*ii*) HHAB estimated the exposure to CPF in drinking water using residue data from PDP or CDPR 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.

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 CDPR 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, use and application timing. In addition, CDPR 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. In conclusion, the actual exposure to CPF in the California drinking water is likely to be somewhere between the "high-end" exposure scenarios based on the CDPR surface and ground water detections and the scenario based on LOD for CPF-oxon from the PDP monitoring.

The main uncertainties in the risk characterization were:

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

and 20 plasma samples). Consequently, the default uncertainty factor of 10 was used to account for the sensitivity within the human population with respect to RBC AChE inhibition.

(*ii*) An uncertainty factor of 10 was used to protect against CPF neurodevelopmental effects in humans. Evidence from human epidemiological and animal toxicology studies showed associations between fetal and early life exposure to CPF and long-term neurodevelopmental and neurobehavioral effects. Mechanistic studies in animals using pathway-based analyses revealed that CPF irreversibly affected neurogenesis and nervous system development in fetuses as well the developing organisms. In the zebrafish model, CPF also caused irreversible neurodevelopmental and neurobehavioral deficits many of which were unrelated to brain and RBC AChE inhibition. However, sufficient data are not available at this time to establish a human dose-response relationship for neurodevelopmental effects. Based on preliminary estimates of the oral *in utero* PoDs for working memory decrements in children 7 yrs old, the threshold for disruption of the endocannabinoid or serotonergic systems in rats and the active concentration causing cognitive, anxiety and learning deficits in zebrafish, the neurodevelopmental effects could be predicted to occur at doses 3-10 fold lower than AChE inhibition. Consequently, in addition to the intra-species uncertainty factor of 10, the HHAB used an extra 10-fold factor for infants <1 year, children1-12 years and women of reproductive age (13-49 years) to protect against CPF neurodevelopmental effects. As more data become available, we will continue to re-evaluate and solidify our position on risk of CPF-mediated neurodevelopmental toxicity.

(*iii*) For spray drift, the risk from acute (1.5 hour) dermal, inhalation, and non-dietary oral exposures was calculated using the 21-day steadaggrey state dermal, inhalation and oral PoDs for CPF. Assuming that the inhibitory effect of CPF on RBC AChE is cumulative, acute PoDs may not be sufficient for characterizing the AChE inhibition from spray drift subsequent to the dietary exposure in one day. Hence, 21-day steady state PoD values were used to evaluate the risk associated with dermal, inhalation, and non-dietary oral exposures from spray drift. Had acute PoDs beenused instead, the resultant MOEs would have been higher. For example, MOEs for non-dietary oral exposures to children 1-2 years and females 13-49 years based on the acute oral PoD for CPF would have been 6 fold higher.

(*iv*) Drinking water exposure for children 1-2 years was used for an aggregate MOE calculations even though infants <1 year 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 match the population subgroup evaluated for exposure to food and spray drift. Had the drinking water exposure estimates for infants <1 years been used, the drinking water MOEs would be 2-fold higher.

TOLERANCE ASSESSMENT

The tolerance assessment was conducted to estimate the point estimate exposure and risk to a single label-approved commodity with CPF residues at the tolerance. The tolerances for the following commodities were evaluated: apple, banana, bell pepper, broccoli, cabbage, sweet corn, grapefruit, onion (bulb), orange, and strawberry. These commodities were selected because of high consumption rates or high contribution to exposure in U.S. EPA's 2011 preliminary dietary exposure assessment. MOEs were evaluated for the four sentinel populations.

The commodities with the least dietary exposure at tolerance were apple, bell pepper, sweet corn, onion, and strawberry. These exposures resulted in MOEs higher than the target of 100 for all four populations. The MOEs were lower than the target of 100 for one or more population subgroups exposed to a tolerance level of CPF on banana, broccoli, cabbage, grapefruit, and orange.

CONCLUSIONS

The health risk assessment of CPF was carried out for 4 sentinel subgroups of the general population: infants (<1 year old), children 1-2 years, children 6-12 years, and women of childbearing age (13-49 years).

Single-route exposure scenarios were evaluated for children 1-2 years and women 13-49 years under acute conditions associated with spray drift near the application site: (i) dermal exposure through skin contact, (ii) inhalation exposure, and (iii) 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 or subchronic (21-day, steady-state) durations and drinking water acute exposures were also calculated for the 4 population subgroups. Aggregate exposures involving multiple routes were also calculated for children 1-2 years at 10-1000 feet from the CPF application site. These routes included inhalation, skin contact with residues (drift deposition), ingestion of residues by object-to-mouth + hand-to-mouth + 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 margin of exposure 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 yrs: MOEs for dermal and inhalation 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; groundboom and airblast at the application rates of 1, 2, 4, or 6 lb a.i./acre.

Children 1-2 yrs: MOEs for dermal and oral exposures (object-to-mouth and incidential 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 for all distances using an aerial or airblast equipment at application rate of 1 lb a.i./acre.

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 at 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 from the edge if a treated field at 1 lb a.i./acre, 100 feet at 2 lb a.i./acre, and 250 feet at 2.3 lb a.i./acre after aerial application of CPF with an aerial equipment. Mitigation should be considered for children 1-2 years near sites where CPF is applied with aerial equipment, and in conjunction with their potential aggregate exposures.

Dietary Exposure:

Food-only exposure: At the 99.9th percentile, the acute dietary MOEs from exposure to CPF residues in food ranged from 1,374 to 3,127 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 1,040. 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 CDPR's Environmental Monitoring Branch (EMON) surface and ground water program. At the 99.9th percentile, the MOEs were highest for PDP (1571-3970) and lowest for the CDPR surface water (405 – 1,299). All MOEs for acute water-only exposure were greater than the target of 100.

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

Children 1-2 yrs: The acute aggregate MOEs were estimated for all routes, including combined deposition.

For the combined deposition, the risk was calculated using the 21-day steady state dermal, inhalation and oral PoDs for CPF and the acute (1.5 h) dermal, inhalation, and non-dietary oral exposures (Summary Table 1). The acute dietary risk from food-only or drinking water probabilistic 99th percentile exposures was calculated using the acute oral PoD for CPF and the acute oral PoD for CPF-oxon, respectively. The drinking water exposures were based on residues from PDP or the CDPR EMON surface water 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 CDPR's Environmental Monitoring (EMON) surface water 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 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 maindriver when the aggregate MOEs fell below the target value of 100 for children 1-2 years (Summary Table 2).

Summary Table 2. Aggregate MOEs for Children 1-2 years at Various Distances Downwind from Fields Treated with CPF by Aircraft or Helicopter[a]

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)										
1	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	251
		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	212
		CD + I + D + DW-PDP[c]	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[c]	1	43	48	55	68	95	127	193
			2	25	28	32	42	65	98	178
			2.3	22	25	30	39	61	94	171
Bell 205 Helicopter	2	CD[b]	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[c]	1	37	49	65	86	126	192	347
			2	20	27	37	51	85	145	287
			2.3	18	25	34	48	80	140	279
		CD + I + D[c]	1	36	47	62	81	115	169	277
			2	19	26	36	49	80	131	238
			2.3	18	24	33	46	76	127	232

		CD + I + D + DW-PDP[c]	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[c]	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[b]	1	135	160	205	311	597	921	1269
			2	66	78	99	147	282	433	603
			2.3	57	67	85	126	239	370	519
		CD + I[c]	1	33	36	40	47	61	75	98
			2	21	23	26	32	42	54	73
			2.3	17	19	21	26	35	44	63
		CD + I + D[c]	1	32	35	39	46	58	71	91
			2	21	23	25	31	41	52	70
			2.3	17	18	21	25	34	43	60
		CD + I + D + DW-PDP[c]	1	32	35	39	45	58	71	91
			2	21	23	25	31	41	51	69
			2.3	17	18	21	25	34	43	60
		CD + I + D + DW-EMON[c]	1	31	33	37	43	55	66	83
			2	20	22	25	30	39	49	65
			2.3	16	18	20	24	32	41	57
103	15	CD[b]	1	105	170	290	498	733	972	1458
			2	52	83	140	237	340	478	790
			2.3	44	71	119	201	290	418	701
		CD + I[c]	1	26	32	40	48	60	76	109
			2	17	21	27	33	43	56	84
			2.3	13	17	22	27	35	47	73
		CD + I + D[c]	1	25	32	38	46	57	72	101
			2	16	21	26	33	41	54	79
			2.3	13	17	21	27	34	46	69
		CD + I + D + DW-PDP[c]	1	25	32	38	46	57	72	100
			2	16	21	26	32	41	54	79
			2.3	13	17	21	27	34	46	69
		CD + I + D + DW-EMON[c]	1	25	31	37	44	54	67	91
			2	16	20	26	31	39	51	73
			2.3	13	17	21	26	33	44	64

a- From U.S. EPA (2014a): 21-Day steady-state PoDs: Dermal: 134.25 mg/kg/d; Oral: 0.099 mg/kg/d, Inhalation steady: 2.37 mg/m^3

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

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

Target MOE = 100

I. INTRODUCTION

The current Risk Characterization Document addresses potential human exposures from the California use of chlorpyrifos (CPF) as an a.i. in insecticide formulations 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) for which there are tolerances. This California Environmental Protection Agency (CalEPA) Department of Pesticide Regulation (CDPR) risk assessment conducted by the Human Health Assessment Branch (HHAB) to evaluate potential adverse effects from CPF in humans for several reasons, including those that follow: 1) there is risk for neurodevelopmental/ neurobehavioral toxicity from exposures during vulnerable developmental windows in fetuses, infants and children; 2) California must determine exposure due to spray drift since data are lacking for residents who are downwind of applications; 3) ingestion by infants can occur from hand-to-mouth activities, as well as through diet and drinking water in California.

An assessment of the relevance of the Physiologically-Based Pharmacokinetic-Pharmacodynamic (PBPK-PD) model utilized by the U.S. 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. Regulatory Status

I.A.1. United States Environmental Protection Agency: U.S. EPA (2014a) www.epa/gov/pesticides/op.)

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: CPF residential use was decreased by the U.S.EPA due to 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.

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

2007: U.S.EPA RED for CPF was produced (U.S. EPA 2007).

2008: National Resources Defense Council (NRDC) petitioned U.S.EPA to ban CPF for all uses and also prepared a lawsuit.

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

2008: U.S.EPA prepared a report for SAP accepting the epidemiological evidence but left the then current safety standards intact.

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

2010: Columbia researchers invited U.S.EPA to a presentation of their 7 year findings.

2011: U.S.EPA does not further restrict CPF; U.S. EPA Interim Reregistration Elegibility Decision (IRED) released (U.S. EPA 2011a).

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

2014: U.S.EPA IRED released (U.S. EPA 2014a). The safety standards are not altered much but there is much objection from academic institutions, the public and other groups.

Scientific Advisory Panel

The Scientific Advisory Panel has conducted several meetings to analyze the assets and weaknesses of available data and to incorporate the results useful for determining the presence of potential adverse neurodevelopmental effects in infants and children after prenatal exposure to CPF. An initial meeting was held in 2008 to focus on literature associated with CPF effects on women and children (U.S. EPA and /SAP 2008). This was followed by a document entitled: "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" for aggregating human data with other data (U.S. EPA and /SAP 2010) because much of the critical data used for the determination of Points of Departure (PoD) were from human studies (Nolan et al. 1984; Rauh et al. 2011; Rauh et al. 2006; Rauh et al. 2012; Smith et al. 2011).

A proposal was made by DOW AgroSciences to use a pharmacokinetic-pharmacodynamic model (PBPK-PD) developed (Timchalk et al. 2002a; Timchalk et al. 2007; Timchalk et al. 2002b; Timchalk and Poet 2008; Timchalk et al. 2005; Timchalk et al. 2006) for CPF PoD determination in risk assessment. The model, based on quantitative estimates of human AChE inhibition after oral, dermal and inhalation exposure to CPF/CPF-oxon via dietary, water, occupational and residential routes was reviewed by the FIFRA SAP (U.S. EPA and /SAP 2012). The U.S. EPA (2011a, 2014a) used AChE inhibition as the critical endpoint for CPF based on the SAP (2008) TCPy: *"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*." Since then the SAP encouraged the U.S.EPA to evaluate current cholinergic (AChE) and non-cholinergic adverse endpoints, including developmental neurotoxicity and cognitive/behavioral alterations from CPF exposure (U.S. EPA and /SAP 2012).

I.A.2. California Human Health Assessment Branch (HHAB), California Environmental Protection Agency (CalEPA)

CPF is a high priority a.i. for risk assessment because of concerns by HHAB for human neurodevelopmental toxicity that can result from its wide use in California. For details on actions taken by CDPR to regulate CPF see:http://www.cdpr.ca.gov/. CPF has been regulated in California as restricted use material since 2014 (http://www.cdpr.ca.gov/docs/legbills/rulepkgs/14-002/final_text.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.

In 2012-2014, HHAB's residue monitoring program detected illegal CPF residues on the commodities shown in Table 1. A high proportion of samples of cactus (leaves or fruit), litchi, and longan contained illegal CPF residues. Most of these commodities were imported. HHAB does not evaluate dietary exposure from illegal residues; however the high frequency of these detections for CPF heightens the risk of additional exposures not considered in the dietary assessment (Table 1).

Food residue programs such as Pesticide Data Program (PDP) have detected residues on foods that have no registered use of CPF. In 2008-2012 PDP detected illegal CPF residues on catfish, cilantro, cherry tomatoes, green onions, spinach, and five other crops (Duncan et al, 2015; APPENDIX 2).

Table 1. Commodities Sampled by CDPR's Pesticide Monitoring Program Reporting Illegal Residues (2012-2014)[a]

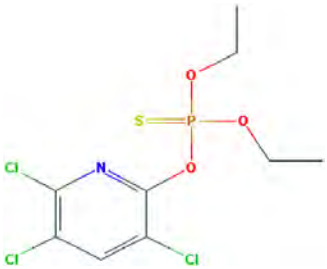
COMMODITY NAME	NUMBER OF SAMPLES TESTED	NUMBER OF SAMPLES WITH ILLEGAL RESIDUES	% WITH ILLEGAL RESIDUES	--- SAMPLES WITH ILLEGAL RESIDUES ---		
				MINIMUM CONC. (ppm)	MAXIMUM CONC. (ppm)	AVERAGE CONC. (ppm)
BEANS, ASPARAGUS	67	1	1.49%	0.66	0.66	0.66
CACTUS, LEAVES OR FRUIT	164	16	9.76%	0.022	0.29	0.093
CARAMBOLA	14	1	7.14%	0.05	0.05	0.05
CELERY	83	1	1.20%	0.02	0.02	0.02
CHINESE AMARANTH	4	1	25.00%	0.03	0.03	0.03
CILANTRO	126	3	2.38%	0.02	0.04	0.033
DILL	5	2	40.00%	0.026	0.075	0.05
LETTUCE, LEAF	121	1	0.83%	0.02	0.02	0.02
LITCHI	19	6	31.58%	0.044	0.21	0.11
LONGAN	21	6	28.57%	0.039	0.2	0.1
PEACH	316	2	0.63%	0.1	0.13	0.12
PEAR	242	3	1.24%	0.059	0.091	0.078
PEPPERS (CHILI TYPE)	211	1	0.47%	1.68	1.68	1.68
SPINACH	409	3	0.73%	0.02	0.09	0.063
SUBTROPICAL & TROPICAL FRUIT (UNSPEC)	15	1	6.67%	0.058	0.058	0.058
SWISS CHARD	31	2	6.45%	0.22	1.29	0.755
TARO	31	2	6.45%	0.032	0.1	0.066
TOMATILLO	301	11	3.65%	0.02	0.15	0.058

a- An illegal residue is one that either exceeds the U.S. tolerance or is detected on a commodity that has no tolerance for the subject pesticide.

I.B. Physical and Chemical Properties

Koshlukova and Reed (2014)

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 g cm ± 3 at 21 °C
Vapor Pressure: 0.00002 mmHg (0.003 Pa) at 25 °C
Boiling Point: >320 °C
Melting Point: 41–42 °C
Flash Point: >200 °F
Conversion Factor: 1 ppm ¼ 14.31 mgm ± 3 at 25 °C
Appearance: Colorless to white, crystalline solid
Odor: Mild mercaptan
Odor Threshold: 0.14 mgm^{-3} (10 ppb).
Solubility in H_2O: <2 mg/L solubility
Organic Solubility: isooctane, methanol
Henry's Law Constant: 0.00001 $atmm^3$ mol/L
Log Koc: 3.73
Kow: 4.8

I.C. Chemical Identification

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 organophosphate (OP) insecticide that was first produced by Dow AgroSciences in 1965. The toxic metabolite CPF-oxon, produced by P450 activation, functions by binding to and then inhibiting acetylcholinesterase (AChE) in the nervous system of a variety of insects (Meister and Sine 2014; U.S. EPA 2014a). CPF is currently used in California on a variety insects found in residential and agricultural scenarios.

I.D. Use and Product Formulations

I.D.1. Uses in California

To date, there are 49 actively registered product labels in California including 4 master labels. 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) Table 2. By law, CDPR requires the

growers and pesticide applicators to report their pesticide use every year through their County Agricultural Commissioners. This pesticide use information can be found in the database named Pesticide Use Reporting (PUR) which is maintained by CDPR and is open to the public (available at: http://www.cdpr.ca.gov/docs/pur/purmain.htm). From the most recent 5 years of PUR data, it can be seen that the total yearly use ranged from 1.10 (low in 2012) to 1.46 (high in 2013) million pounds to 0.9 to 1.3 million acres with the average of 1 lb/acre which basically reflects the median application rate based on the label. There were no obvious trends in yearly use or acres treated which fluctuated year to year but use was at its highest in the most recent year reported (2013). The crop treatment data show that almonds received the highest poundage of CPF (range: 192,482 in 2012 to 448,673 lb in 2013) compared to other crops.

Table 2. Pesticide Use Data for CPF in California from 2009-2013

Year	Total yearly use (lb)	Total yearly treated (acre)	Top 5 crops treated	Yearly use for top 5 crops (lb)
2009	1,235,481	919,402	Almond	330,409
			Walnut	177,430
			Alfalfa	171,452
			Orange	119,228
			Grape, wine	94,647
2010	1,285,630	1,095,218	Almond	262,002
			Alfalfa	175,834
			Walnut	171,422
			Orange	171,030
			Cotton	115,024
2011	1,296,074	1,186,979	Almond	231,067
			Orange	205,595
			Cotton	194,173
			Alfalfa	185,879
			Walnut	163,097
2012	1,100,873	1,051,292	Almond	192,482
			Walnut	174,931
			Alfalfa	174,669
			Orange	129,546
			Cotton	97,769
2013	1,460,672	1,288,690	Almond	448,673
			Alfalfa	193,653
			Walnut	166,208
			Cotton	157,790
			Orange	152,324

I.D.2. Technical and Product Formulations

CPF (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate (CAS number:2921-88-2 and CDPR chemical code:253). It is the a.i. 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. To date, there are 49 actively registered product labels in California including 4 master labels.).

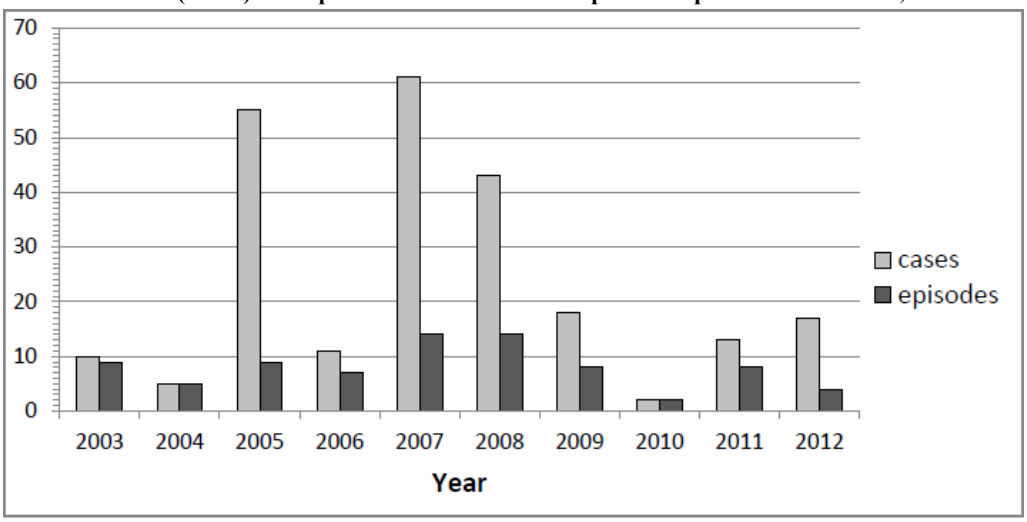
I.E. Human Illness Reports

This evaluation used only CDPR's Pesticide Illness Surveillance Program (PISP) database for human incident data on CPF (CalEPA 2015). This database, though specific to California, contains all illness and injury reports potentially related to pesticide exposure and may provide patterns or trends

associated with the use of a particular pesticide. PISP defines a "case" as a pesticide exposure and its apparent effects on one individual's health and "episode" as an incident in which one or more people experience pesticide exposure from a particular source with subsequent development or exacerbation of symptoms. Occasionally, a single episode can give rise to a large number of cases. Cases are classified by the relationship of the exposure to a specific pesticide and subsequent effects. A **"**definite" relationship indicates that both physical and medical evidence supported a direct causal link between an exposure and its subsequent health effects. A "probable" relationship indicates that limited or circumstantial evidence supported a similar causal link. A "possible" relationship indicates that health effects generally corresponded to the reported exposure, but evidence was not available to support a causal link.

In California from 2003 to 2012, 235 illness cases (72 episodes) involving CPF were reported (Figure 1).

Figure 1. Numbers of Illnesses (Cases) and Episodes Related to CPF Exposure Reported in California, 2003-2012



a- California PISP report generated for pesticide active ingredient CPF, years 2003-2012 (CalEPA 2015)

The type of exposure is also documented in the PISP report. Drift exposure includes spray, mist, fumes or odor carried from the target site by air. Residue exposure involves a pesticide that remains in the environment for a period of time following an application or drift. Other types of exposure include ingestion (oral), spills and contact during clean-up, and direct sprays. Table 3 summarizes the distribution of exposure types of the 235 CPF-related cases from 2003 to 2012. Drift and residue exposures contributed to the majority of the reported cases.

Table 3. Exposure Types of CPF-Related Cases (2003-2012)

Exposure Type	Cases
Drift[a]	154
Residue[b]	43
Direct Spray/Squirt[c]	6
Spill/Other Direct[d]	5
Ingestion[e]	6
Other[f]	6
Unknown	15
Total	235

a-Spray, mist, fumes, or odor carried from target site by air
b-Pesticide remaining in the environment following application or drift

c-Material propelled by the application or mix/load equipment.
d-Contact made during application or mixing/loading which is not propelled, expected contact during use (cleaning), or leaks/spills not related to application
e-Intentional or unintentional oral ingestion
f-Indirect contact not related to application, exposure to smoke or fire involving the pesticide, or transfer (e.g. glove to eye)

The PISP database also contains information about the nature of the pesticide exposure and the subsequent illness or injury. Illnesses are characterized based on types of symptoms described: systemic (symptoms such as headache, confusion, salivation, dizziness, and nausea); skin (symptoms such as irritation, itching, and rashes); eye (symptoms such as irritation and burning); respiratory (symptoms such as airway irritation, wheezing, and shortness of breath). Illness reports associated with exposure to CPFfrom 2003-2012 are summarized in Table 4. Irritated airways and systemic effects such as dizziness, nausea, and headache were the most frequently reported symptoms related to CPF exposure (CalEPA 2015).

Table 4. PISP Reported Symptoms Related to Chlorpyrifos (2003-2012)

Illness Type	Alone [a]	In Combination [b]	Total
Systemic only	21	61	82
Systemic & Eye	4	5	9
Systemic & Respiratory	11	21	32
Systemic & Skin	13	5	18
Systemic, Respiratory, Eye	8	12	20
Systemic, Respiratory, Skin	10	1	11
Systemic, Skin, Eye	3	0	3
Systemic, Respiratory, Skin, Eye	5	1	6
Respiratory only	9	15	24
Respiratory & Eye	5	1	6
Skin only	8	4	12
Eye only	4	3	7
Other combinations of types [c]	3	2	5
Total	104	131	235

a- Chlorpyrifos was applied as a sole active ingredient.
b- Chlorpyrifos formulated in a product with other pesticides.
c- Includes 3 less common combinations of eye, skin, respiratory, and effects.

According to the California Pesticide Illness Query (CalPIQ; http://apps.cdpr.ca.gov/calpiq/), CPF-related illnesses represented, on average, 2% of the total yearly reported pesticide illnesses: 17/981, 13/1013, 2/793, 18/1007, and 43/895 for the years 2012, 2011, 2010, 2009, and 2008, respectively.

I.F. 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 interation 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. CPF hydrolysis produces O-ethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate or 3,5,6-trichloro-2-pyridinol (TCPy) and phosphorthioic acid 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 absorbs strongly in soil and resists leaching to groundwater. CPF will persist for weeks or months in indoor environments (Berkowitz et al. 2003; Rauh et al. 2006; U.S. EPA 2014a). In the environment CPF is oxidized to the toxic metabolite CPF-oxon by photolysis, aerobic metabolism, and chlorination (e.g., drinking water). CPF-oxon is rapidly hydrolyzed to TCPy and its glucuronide conjugates. The CPF K_{ow} (4.8) indicates a potential for bioaccumulation in aquatic (TCPy and conjugates detected in fish tissues) and terrestrial food chains.

II. TOXICOLOGY PROFILE

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 studies contributing to the hazard assessment can be found in the HHAB Summary of Toxicology Data APPENDIX 1) and in the U.S.EPA Interim Re-registration Eligibility Decision documents (IRED) (U.S. EPA 2011a, 2014a).

II.A. Acetylcholinesterase Inhibition

AChE normally breaks down the neurotransmitter, acetylcholine (ACh), at a central nervous system (CNS) synaptic cleft or at neuromuscular or neuro-glandular junctions in the peripheral nervous system (PNS; Figure 2) (Casida and Quistad 2004; Testai et al. 2010). When AChE inhibition occurs, ACh accumulates and causes unremitting nerve impulses that lead to continuous muscle responses in the peripheral nervous system (PNS) or neural stimulation in the central nervous system (CNS).

The active CPF metabolite is CPF-oxon which inhibits AChE by binding at the active site. This risk assessment will focus only on effects reported after treatment with the parent active pesticidal ingredient (i.e. CPF). ChE occurs in plasma as BuChE, in red blood cells only as AChE and in brain primarily as AChE (Eaton et al. 2008; Testai et al. 2010). In rat brain AChE activity is higher (90% of total) compared to BuChE activity (10%) (Li et al. 2000a; Mortensen et al. 1998). The BuChE:AChE ratio varies with species and is 1000:1 in humans, 2:1 in female rats and 7:1 dogs, but 1:3 in male rats (Brimijoin 1992; Scarsella et al. 1979).

In general, HHAB considers brain ChE inhibition to be indicative of overt toxicity since it is one of the primary functional target sites and more subtle central neurological signs, such as memory and learning losses, may not be easily detected in animals unless they are specifically tested for these effects. The toxicological significance of plasma and RBC AChE inhibition is less certain because the physiological function of ChEs in blood have not been clearly established, although several possible physiological functions have been proposed. Plasma ChE, or more specifically BuChE, may be involved in the binding/metabolism of certain drugs, such as succinylcholine, which suggests that its inhibition may compromise an organism's ability to defend against subsequent toxic insults (Lockridge and Masson 2000). BuChE is also the predominant form of ChE 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 including 1) substrate inhibition of AChE at high ACh concentrations, 2) the survival of AChE knockout mice, and 3) the increase in BuChE levels with Alzheimer's disease as AChE levels decrease (Ballard and Perry 2003; Giacobini 2003; Li et al. 2000a).

Although blood ChE (plasma BuChE and RBC AChE) inhibition is not usually detrimental, it can be used as a surrogate for brain and/or peripheral AChE inhibition when such data are lacking (U.S. EPA 2000b). For example, plasma/BuChE in humans, inhibited up to 85%, may not lead to clinical signs but can serve as an indicator of brain or peripheral AChE inhibition (Nolan et al. 1984). RBC AChE inhibition values are generally preferred over BuChE because RBCs contain only AChE whereas plasma can contain both contains BuChE and AChE) and the ratios of these two enzymes vary depending on the species (Testai et al. 2010). This is important in no-observed-effect-level (NOEL) or point-of-departure (PoD) determinations because the test compound (e.g., CPF) may have considerably different affinity for the active site of BuChE versus AChE (U.S. EPA 2000b).

The Joint Meeting on Pesticide Residues of the WHO/JMPR (1999) concluded that RBC AChE activity should only be used as a surrogate for peripheral ChE activity at the time of peak effect with acute exposure since RBCs lack the ability to synthesize new AChE (Brimijoin 1992). 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. HHAB is currently reevaluating the use of ChE inhibition data in its risk assessments. In anticipation of changes in the use of these endpoints in the risk assessments, NOELs for blood and brain inhibition were identified in this document based on statistical significance.

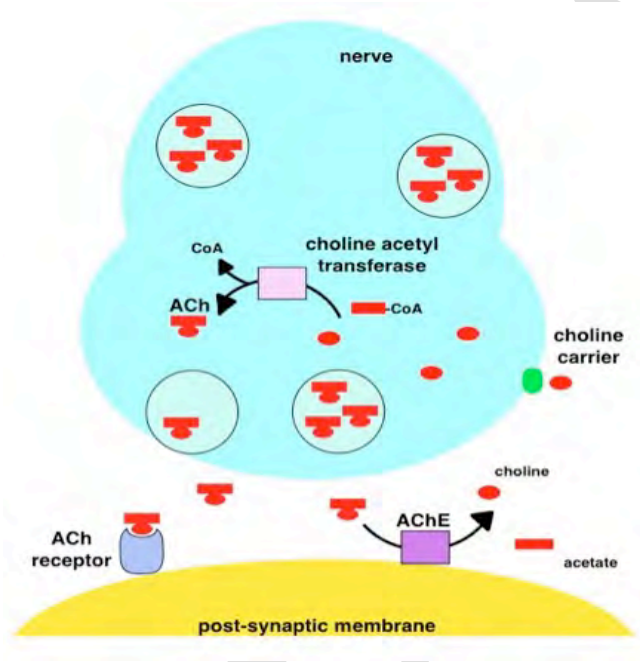


Figure 2. Acetylcholinesterase (AChE) is in cholinergic neurons at nerve-nerve central nervous system (CNS) and neuromuscular (PNS) junctions.
AChE breaks down acetylcholine (ACh) thereby ending its action at the synapses between neurons and between neurons and muscle fibers or glands. Inhibition of AChE leads to an accumulation of ACh and results in prolonged stimulation. In the PNS the ACh accumulation results in "cholinergic" responses such as smooth muscle contractions (e.g., abdominal cramps), glandular secretions (e.g., sweating), skeletal muscle twitching, and paralysis (Available https://en.wikipedia.org/wiki/Acetylcholinesterase).

II.B. Metabolism and Pharmacokinetics

Numerous articles have described the metabolism of CPF in animals and in humans (Eaton et al. 2008; Testai et al. 2010; Timchalk et al. 2002a; Timchalk et al. 2002b; Timchalk and Poet 2008; Timchalk et al. 2005; Timchalk et al. 2006). CPF-oxon is formed when CPF is metabolized by P450 (CYP1A2, 2B6, 2C19, 3A4, 3A5, and 3A7) (Foxenberg et al. 2011; Testai et al. 2010; Timchalk et al. 2002b). Subsequently the CPF-oxon (which is unstable) is degraded by a host of enzymes including B-esterases (BuChE and CES) and the calcium-activated A-esterases (PON1), found in blood, brain, liver and other tissues (Figure 3) (Testai et al. 2010). These enzymes detoxify CPF-oxon before it can inhibit 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 can serve as a biomarker for CPF metabolism (Testai et al. 2010).

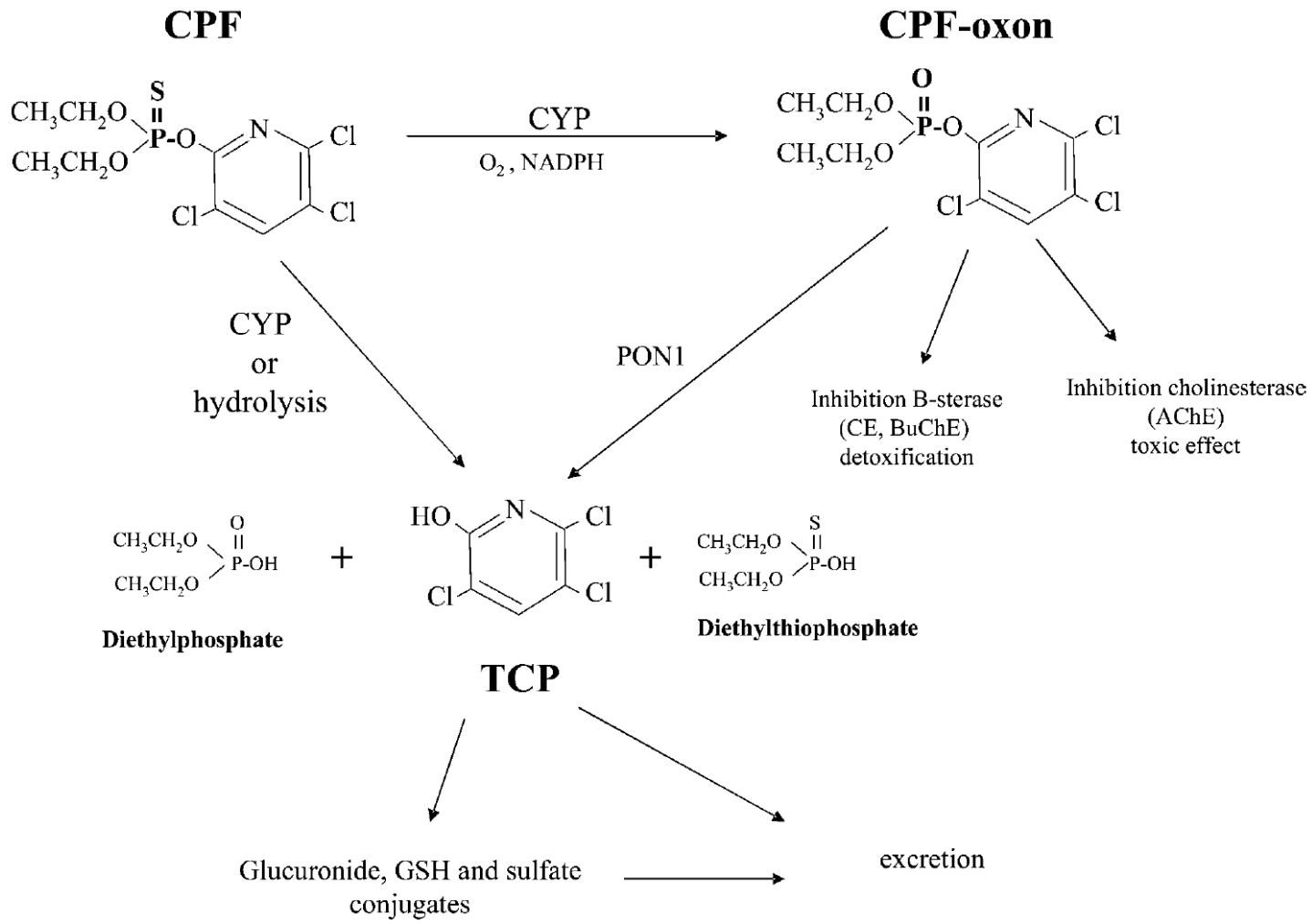


Figure 3. The major metabolic pathway for CPF (Testai et al. 2010)

II.B.1. Metabolism and Pharmacokinetics in Rat

Nolan et al. (1984): ^{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 at 0.5 or 25 mg/kg or 15 consecutive daily doses of unlabeled CPF at 0.5 mg/kg/d, followed 1 day after the 15th dose with a single labeled dose of 0.5 mg/kg. The TCPy group in the CPF molecule was radiolabeled. 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 dose, 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 ($T_{1/2}$ 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 composed chiefly of TCPy, and usually slightly more of it as the glucuronide conjugate, collectively accounting 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 label was obtained within the first 24 hours. Exhaled CO_2 was trapped for radioanalysis from the 25 mg/kg group which 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). This study was submitted by the registrant.

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 erythrocyte 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 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 and 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 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 erythrocyte AChE demonstrated by one of the subjects in the 2.0 mg/kg treatment group).

Part 2: As a continuation of the above study, 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 (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 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 to 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. Thus this study shows 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 and 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, urine samples up to 120 hours post-dosing. Most of the treatment dose was "wash-off" from the skin (42%–67%). At 5 mg and 15 mg CPF, the urinary TCPy was 131.8 µg or 115.6 µg, respectively, 120 hrs post-dosing. Approximately 4.3% of the applied dose was absorbed in both doses as indicated by the lack of significant increase in urinary TCPy (115.6 µg) from the low to high dose. This indicates that the higher dose did not result in an increased absorption, when compared to the lower dose (i.e. "percutaneous penetration rate was constant"). CPF clearance was not finished by 120 hours and therefore CPF or TCPy was likely retained in the skin and/or various body compartments. The elimination t½ was 41 h and therefore repeated occupational exposure may result in accumulation of CPF and/or its metabolites.

II.B.2.d. Metabolism of CPF by Human P450 Isoforms

CPF is both activated by cytochrome P450 (CYP) through desulfuration to form CPF-oxon and degraded by CYP through dearylation (Tang et al. 2001). In this study human liver microsome (HLM) CYP isoforms (expressed in human lymphoblastoma cells) were used to show that CYP1A2, 2B6, 2C91, 2C19, and 3A4 are involved in CPF metabolism. CYP2B6 has the highest desulfuration activity and the greatest dearylation activity is from 2C19. CYP3A4 has high activity for both dearylation and desulfuration. Based on these results, HLM CYP phenotype profiles for individuals can be used to predict the metabolic activation or deactivation of CPF depending on their CYP2B6, 2C19, and 3A4 levels in microsomes. For example HLM CYP phenotypes with high CYP2C19 but low 3A4 and 2B6 are more active in dearylation than in desulfuration. Persons with high CYP2B6 and 3A4 are most likely to form CPF-oxon. These data indicate that there are different sensitivities among individuals based on their P450 phenotype. In addition, this study reported gender differences in metabolism with female HLM having greater activity than males.

II.B.3. PBPK-PD Modeling Reported in the U.S.EPA IRED (U.S. EPA 2014a)

A beneficial trend in risk assessment is the use of the physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) model developed initially by Gearhart et al. (1990), Timchalk et al. (2002a); Timchalk et al. (2002b). For CPF the model is based on 10% inhibition of RBC AChE after an acute (single day, 24 hr) or steady-state (21-d) exposure of CPF in humans (Kisicki et al. 1999; Nolan et al. 1984). 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 (Lowe et al. 2009; Poet 2015; Poet et al. 2014; Poet et al. 2003; Smith et al. 2014; Smith et al. 2011; Timchalk et al. 2007; Timchalk and Poet 2008) 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). Table 5 illustrates the measured data used for the PBPK-PD model validation. 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 (Poet et al. 2014; Timchalk and Poet 2008). Note that some parameters are obtained by use of animal data.

Table 5. Data Concordance and Completeness for PBPK-PD Model Validation

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

a-Yellow highlighted area indicates measured data used for the PBPK-PD model validation that was the most complete and showed the best concordance for RBC AChE and BuChE inhibition and TCPy biomarkers for oral, dermal and inhalation routes of exposure (Poet et al. 2014; Timchalk and Poet 2008).

III.B.3.a. PBPK-PD Model Predicts Inter-individuality and Susceptibility to CPF Effects

CPFoxon metabolism produces the biomarker of detoxification: TCPy. The ratio of CPF-oxon to TCPy varies with species, gender, age, P450 enzyme profiles, and P450 enzyme polymorphisms (Ma and Chambers 1994). The forms of human P450s that metabolize CPF are CYP2B6 (desulfuration), CYP2C19 (dearylation), and CYP3A4/5 for both pathways (Buratti et al. 2003; Mutch and Williams 2004; Tang et al. 2001). The three main P450 enzymes associated with CPF metabolism (CYP2B6, 2C19, and 3A4) were shown to follow different age-dependent expression patterns and variability in humans due to changes in regulatory mechanisms and polymorphisms (Croom et al. 2009). CPFoxon is also metabolized by PON1 (hepatic and extrahepatic A-esterases, CPFoxonase) (Pond et al. 1995), and BuChE (plasma ChE or B-esterases: Figure 1) to form TCPy and diethylphosphate (Timchalk et al. 2002b). PON1 (genotypes QQ, QR, RR) (Costa and Furlong 2010) activities involved in age-related CPF-oxon metabolism and the predicted effect on AChE inhibition are incorporated into the current PBPK-PD model. PON1 genetic polymorphisms account for 40-fold variation in plasma activity (Costa and Furlong 2010; Costa et al. 2013) and the activity in cord blood ranged 34-fold in neonates (lower in adults) (Huen et al. 2010). The enzyme activity (catalytic efficiency) of $PON1_{192}$ R alloform is greater than that of the $PON1_{192}$ Q alloform in degrading CPFoxon (Li et al. 2000b). Human PON1 activity is low *in utero* (24% lower at 33-36 weeks gestation) than at birth (Ecobichon and Stephens 1973) but it is also low at birth compared to adults (shown to plateau at 6-15 months) (Chen et al. 2003; Cole et al. 2003; Ecobichon and Stephens 1973; Mueller et al. 1983). Low PON1 *in utero* and at a young age can affect CPF toxicity to fetuses, neonates and young children. Inter-individuality for the esterases and P450s was accounted for in the PBPK-PD model by methods described in Smith et al. (2011). .*In vitro* data obtained from human tissues and plasma from neonate through adult analyzed by probabilistic distributions for age-related effects to PON1, P450 activation to oxon and detoxification to TCPy.

CPF metabolism differs with age and this must be quantified when constructing a PBPK-PD model for CPF Smith et al. (2011). CPF age-related metabolism was assessed by quantifying *in vitro* metabolite formation by hepatic microsomes from human corpses aged 13 days to 75 years and from the plasma of humans aged 3 days to 43 years. CPF is metabolized in the liver via cytochrome P450 to form CPF-oxon and 3,5,6-trichloro-2-pyridinol. The V_{max} values for these metabolic processes were 0.35 ±0.21 and 0.73 ± 0.38 nmol/min^{-1} /mg microsomal protein^{-1} (mean ± S.D.), respectively. Mean (±S.D.) hepatic metabolic conversion of CPF-oxon hydrolysis (CPF-oxonase) V_{max} was 78 ± 44 nmol/min^{-1}/mg microsomal protein^{-1}. Based on these results no age-dependent relationships (per microsomal protein by linear regression models) occurred. CPF bioactivation to detoxification ratios (V_{max}) did not differ across age groups. Plasma CPF-

oxonase and total plasma protein levels showed age-dependent increases (per volume of plasma). CPF-oxon hydrolysis V_{max} for children (<6 mos) and adults (>16 years) were 1900 ± 660 and 6800 ± 1600 nmol/min^{-1}/ml^{-1}, respectively (Mean ± S.D.), which, according to authors "at environmental exposure levels, this high-capacity enzyme is likely to be sufficient even in infants." 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.

Findings from the Smith et al. (2011) study were then applied to a new model incorporating life-stage changes on human metabolism of CPF to CPF-oxon and TCPy as well as RBC AChE inhibition (Smith et al. 2014). These life stage changes included effects on human anatomy, physiology, pharmacokinetics and pharmacodynamics. It also incorporated changes in body weight, organ volumes, and metabolic rates based on available literature that were then mathematically tested against controlled adult human exposure studies (Nolan et al. 1984). Results showed that at high acute oral doses (>0.6 mg/kg/d) children age 6 are predicted to have higher levels of CPF-oxon in blood, resulting in higher RBC AChE inhibition compared to adults. However at doses of 0.0006-0.006 mg/kg/d adults are predicted to have higher levels of CPF-oxon and increased RBC AChE than children Figure 5.

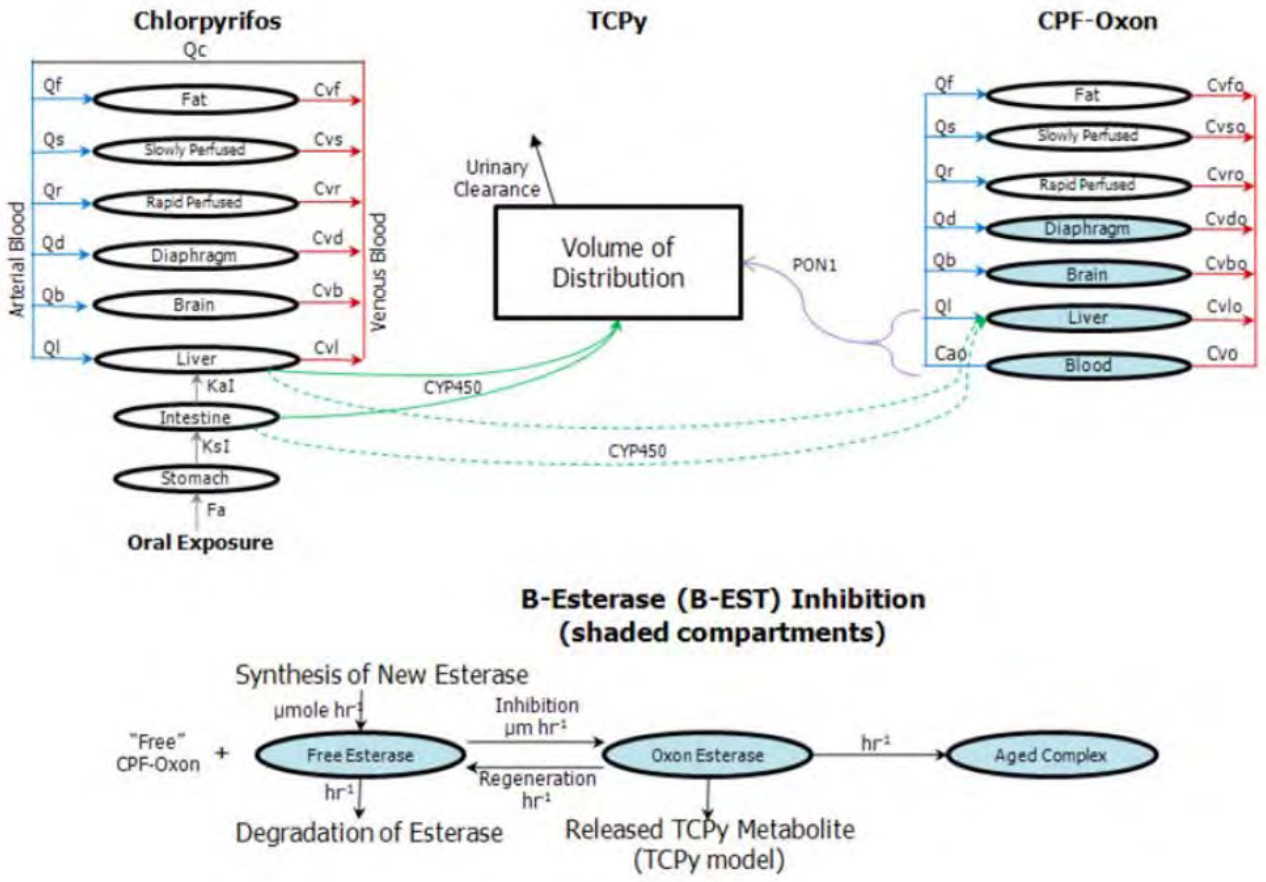


Figure 4. PBPK-PD Model (typical adult) structure
The shaded compartments denote tissues which contain B-esterases (BuChE, CES: bottom panel). Tissue volumes and enzyme activities (V_{max}) change with age based on liver and/or blood compartmental growth (From U.S. EPA (2014a)).

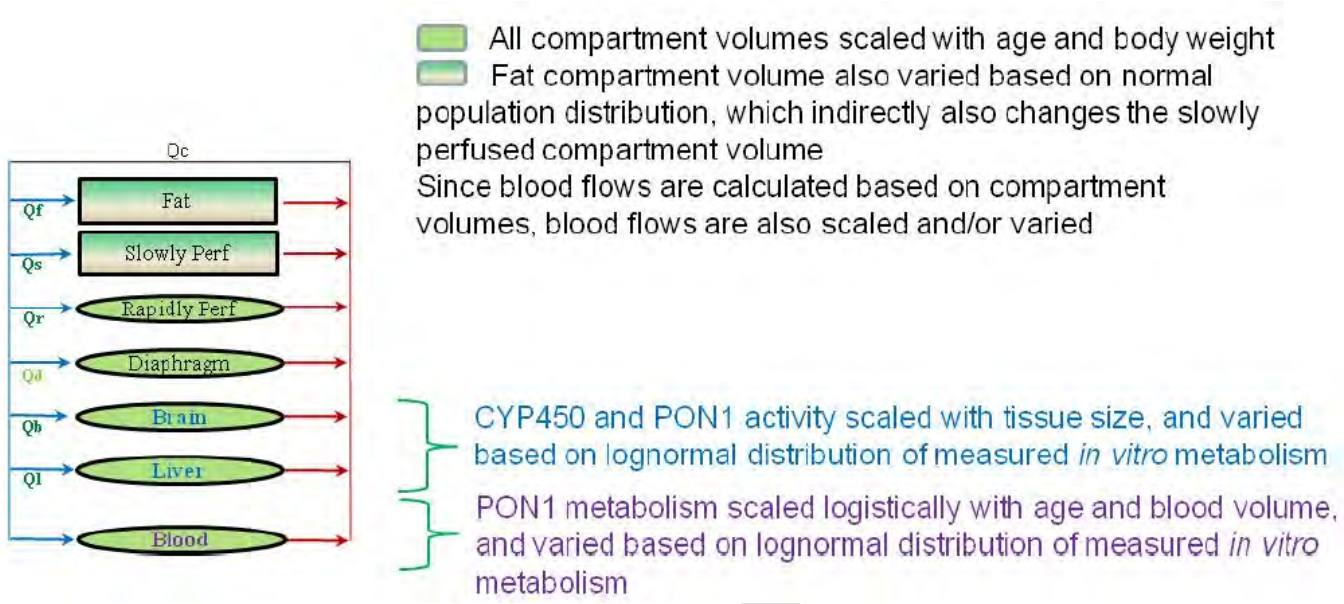


Figure 5. Schematic of age and body weight dependences in the PBPK-PD model.
All compartment volumes and blood flows vary with age and body weight. *In vivo* metabolic rates are scaled based on tissue size (measured *in vitro* values scaled to describe tissue-specific (brain, blood, and liver) metabolism); in blood, PON1 metabolism of oxon is not only blood volume but also age-dependent (From U.S. EPA (2014a)).

The PBPK-PD model also includes predicted pregnancy-related changes to CPF and CPFoxon metabolism and disposition which are influenced by maternal and fetal physiological and pharmacokinetic modifications during pregnancy (Abduljalil et al. 2012; Lu et al. 2012). Poet (2015) developed a version of the PBPK-PD model that encompasses these changes for oral, dermal and inhalation exposures and supsequent CPF and CPFoxon-related RBC AChE inhibition in humans. This model was designed to rationalize the removal of the 10x FQPA safety factor that was added by the U.S. EPA (2011a, 2014a) based on unknown physiological or pharmacodynamics of RBC AChE during pregnancy. Many of the aspects of the multi-route and multi-lifestage model (e.g., tissue growth over the course of life span) served as the foundation for the pregnancy model, including placental and fetal growth (Poet et al. 2014). Pregnancy specific changes such as fat and fat free mass increases and changes in metabolic rates were incorporated into the model (Abduljalil et al. 2012; Lu et al. 2012). Model changes consist of (taken from Poet (2015):

- Uterine, placental and fetal compartments, which grow over the course of pregnancy.
- Pregnancy specific changes in the slow compartment, fat, and rapid compartments.
- Pregnancy specific changes in blood composition
- Changes in blood composition result in increased blood volume, decreased hematocrit
- Lipids, triglycerides, and cholesterol increase – leads to changes in partitioning
- Pregnancy specific changes in metabolism
- CYP450s – some increase, some decrease. Evidence for changes based mostly on clearance of marker substrates

Poet (2015) made comparisons between pregnant and non-pregnant women by using Monte Carlo distributions (including DDEFs) to simulate human variability in response to CPF oral and dermal exposures. Results show that during pregnancy circulating CPF is decreased and CPFoxon is increased, when compared to non-pregnant women, especially at high doses (≥0.5 mg/kg). RBC AChE inhibition occurs at doses that are 3-20% less than for non-pregnant women as previously predicted in the life-stage PBPK-PD model (Poet et al. 2014). The most effective dose of CPF resulting in 10% RBC AChE inhibition (ED_{10}) is equivalent between pregnant and non-pregnant women and DDEF are consistent for all simulated populations. The PBPK-PD pregnancy model also shows 10% inhibition of RBC AChE occurring at 0.1-1.0 mg/kg/d for oral, 10-150 mg/m^3 for inhalation (2 hr acute; 2 hr/d, 21-d) and 10-150 mg/kg/d for dermal (4 hr acute; 4 hr/d, 21-d). The range indicates ~10% RBC AChE inhibition at steady-state (low value) and acute (high value). Their final conclusion was that a DDEF (extrapolation, or uncertainty factor) of 4x (protects >99% of the population) was sufficient to protect males and females, non-pregnant women and pregnant women (basically all cohorts) from dermal and oral CPF exposures.

II.C. Acute Toxicity

The profile of acute CPF toxicity has been extensively described and reported by others (Eaton et al. 2008; Testai et al. 2010; U.S. EPA 2007, 2011a, 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 HHAB by registrants (see APPENDIX 1for HHAB one-liners) 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}s; dermal and eye irritation, dermal sensitization and acute delayed neurotoxicity studies using technical CPF and required for CPF registration were submitted by the registrant (Table 6). Rat had primarily Category II for oral and dermal and Category II/III for inhalation. However rabbit was not sensitive to CPF dermally administered and had slight/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 hours)	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. U.S. 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 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 and to humans in order to assess AChE-related effects. Treatments 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 (APPENDIX 1) (U.S. EPA 2007, 2011a, 2014a). NOELs and LOELs are included. Many of the studies reported below will be discussed in more detail in later sections which cover effects from CPF on development, reproduction and developmental neurotoxicity. The information was divided in this manner because in most acute CPF studies the predominant effects were due to AChE inhibition. However, CPF also had profound effects on aspects of development that needed to be highlighted in a separate category for the purpose of hazard identification.

Table 7. ChE Inhibition with Acute or Short-Term Exposure to Chlorpyrifos and the Respective NOELs and LOELs

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[a]
Oral Gavage or Subcutaneous Treatment to Pup/Neonate					
Rat SD M/F	Gavage c.o. or milk[d] PND 11	At 6-8 hr: ↓RBC, Plasma & BrChE	**F: plasma, RBC, Brain 0.5[b]**	2	1
Rat SD M/F	Gavage c.o. PND 11-21	At 10 days (6 hr): ↓RBC, Plasma & BrChE	**M &F: plasma: 0.05; RBC: 0.1; brain: 0.5**	1	1
Rat SD M/F	Gavage c.o. PND 10-16	At: 4 hr: ↓Forebrain, Medulla-Pons and Plasma ChE	--	1.0	2
Rat SD M/F	Gavage c.o. PND 10-16	At 4, 12, 24, & 48 hr: ↓Forebrain AChE	--	1.0	3
Rat SD M/F	Gavage c.o. PND 10-16	At 4-10 hr: ↓Plasma ChE	--	0.5	4
Rat M	Gavage c.o.PND 17	At 4 hr: ↓brain and whole blood ChE	**BMDL (U.S.EPA, 2014) 0.43**	BMD (U.S.EPA, 2014) 1.54	5
Rat SD M/F	Gavage c.o. PND 1	At 12 hr: ↓forebrain AChE	--	1.5	6
Rat SD M/F	Gavage c.o. PND 5, 12, 17	PND 5, 12, 17 at 3, 6 & 24 hr, respectively: ↓RBC, Plasma & BrChE	--	1.0	7
Rat SD M/F	Gavage c.o. PND 1-6 Tested PND 4, 7, 12	All time points: ↓ forebrain AChE	--	1.5	6
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 (excluding cerebellum and medulla-pons) AChE	--	1.5	8
Rat SD M/F	Gavage c.o. PND 1-4 or 1-8	PND 1-4 (4 hr): ↓ brain AChE	--	1.0	9
Rat SD M/F	Gavage Peanut Oil PND 7 or PND 7-20	Pup & adult: acute & 13 d at 4 hr: ↓RBC, Plasma & Frontal cortex ChE	**Pup Acute: 0.15 Pup Repeated: 0.75**	Pup Acute: 0.75 Pup Repeated: 1.5	10
Rat ? M/F	s.c. DMSO PND 1-4	At 24 hr:↓Brainstem AChE	--	1.0 LDT	11
Rat SD M/F	s.c. DMSO PND 1 (1 dose/1 exposure)	At 2 hr:↓brainstem, cerebellum and forebrain AChE	--	1.0 LDT	12
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): ↓RBC, Plasma, forebrain, hindbrain & heart ChE Pup: ↓RBC, Plasma, forebrain, hindbrain & heart ChE	Dam: Plasma & RBC <0.3 LDT; Brain: 0.3 Pup: 1.0	Dam: 0.3 Pup: 5.0	13
Rat F-344 F	Gavage c.o. GD 6-15	At GD 21: ↓ plasma and RBC AChE	**0.1**	3.0	*14
Rat CD F	Gavage c.o.GD 6-15	At GD 20:↓ plasma ChE (only AChE tested)	--	0.5	*15
Rat Crl:CD7(SD)BR VAF/Plus F	Gavage c.o. GD6-LD 11	LD 22: ↓RBC, Plasma & BrChE	**Dam: --**	Dam: 0.3	16
Rat SD F	Gavage c.o. GD6-20	GD 20 ↓RBC, Plasma & BrChE	**NOEL Plasma & RBC <0.3 LDT; Brain: 1**	LOEL Plasma & RBC 1.0 Brain: 5	17
Mouse CF-1 F	Gavage cottonseed oil GD 6-15	At GD 18: ↓ plasma and RBC AChE	--	1.0	*18
Rabbit HY/CR-NZW	Gavage c.o. GD 7-19 Plasma ChE only	At GD 17d:↓ plasma ChE	--	1.0	*19
Rat SD M/F	s.c. DMSO GD 9-12 or GD 17-20	At GD 21: ↓Brainstem & forebrain AChE	--	1.0	20
Adult Treatment					
Rat SD M/F	Gavage c.o. 10 d	Day 10 (6-8 hr):↓RBC, Plasma & BrChE	**F only: plasma &RBC: 0.1; Brain: 1**	3.5	1
Rat SD M	Gavage Peanut Oil Adult at PND 70 or 70-83	Pup & adult: acute & 13 d at 4 hr: ↓RBC, Plasma & Frontal cortex ChE	**Acute: 0.75 Repeated: 0.15**	Acute: 1.5 Repeated: 0.75	10
Rat SD F	Gavage c.o. or diet[d] Single dosing	At 8 hr: ↓RBC, Plasma & BrChE	**1 dosing: plasma = 0.05; RBC = 0.1; brain AChE = 0.5 mg/kg [c]**	plasma & RBC = 0.5 brain 2.0	1
Mouse C57Bl/6J M	s.c. DMSO; 1d or 5d Brain AChE only	At 3-6 hr 1 injection: No brain AChE effects 3-24 hr 5 injections: ↓brain AChE	--	LOEL 5.0	21

Table 7. ChE Inhibition with Acute or Short-Term Exposure to Chlorpyrifos and the Respective NOELs and LOELs

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[a]
Human M	1dose (methylene chloride on a 0.5-g lactose tablet) [d]	At 1-30 d: No significant effect on plasma ChE	Plasma ChE: >0.5 mg/kg (Only dose tested)	--	22
Human M/F	Powder in gelatin capsule[g]	At 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours post dose. ↓RBC AChE (1 subject)	1.0 mg/kg	2.0	23
Dermal Treatment					
Rat F344 F	Dermal c.o. 6 hr/d 4d Probe Study	↓Plasma and RBC AChE	1.0 mg/kg	10	24
Human M	1 exposure; dissolved in methylene chloride	No significant effect on plasma ChE	>5 mg/kg (1 dose tested)	--	25
Inhalation Treatment					
Rat Crl:CD (SD) M/F	Aerosol Nose Only; 2-6 hrs	↓ Plasma and lung AChE; ↓ RBC and brain AChE	AChE $BMDL_{10}$ 1.31 mg/m^3 (0.89 mg/kg/d) 0.09 ppm[f]	AChE BMD_{10} 3.17 mg/m^3 0.22 ppm[f]	26
Rat CD(SD): Crl F	Vapor Nose Only; single dose	No significant effects on plasma, RBC or brain ChE	17.7 ppb (0.254 mg/m^3) only dose tested	No LOEL	27
Rat F-344	Vapor Nose only or Whole Body 6 hr	↓ plasma ChE in whole body exposure (attributed to oral ingestion or dermal exposure)	3.5 ppm (50.1 mg/m^3)	6.0 ppm (100.2 mg/m^3)	28

a- 1. Marty et al. (2012), Marty and Andrus (2010); 2. Carr et al. (2011); 3. Carr et al. (2013); 4. Carr et al. (2014); 5. Moser et al. (2006); 6. Betancourt and Carr (2004); 7. Timchalk et al. (2006); 8. Richardson and Chambers (2005); 9. Guo-Ross et al. (2007); 10. Zheng et al. (2000); 11. Song et al. (1997); 12. Dam et al. (2000); 13. Mattsson et al. (2000) ; 14. Ouellette et al. (1983); 15. Rubin et al. (1987a); 16. Hoberman (1998); 17. Maurissen et al. (2000); 18. Deacon et al. (1979); 19. Rubin et al. (1987b); 20. Qiao et al. (2002); 21. Speed et al. (2012); 22. Nolan et al. (1984); 23. Kisicki et al. (1999); 24. Calhoun and Johnson (1988); 25. Nolan et al. (1982) and Griffin et al. (1999); 26. Hotchkiss et al. (2010); 27. Hotchkiss et al. (2013); 28. Landry et al. (1986)
b- Milk and corn oil results were the same for males and females except brain AChE with milk: NOEL: 2.0 M and 0.5 F
c- Results were the same with diet and gavage
d- Single exposure
e- Conversion to mg/kg/d: 3.7 mg/m^3 x 0.96 mg/m^3 (breathing rate in rat) x 0.25 (treatment: 6 hr/24 hr) = 0.89 mg/kg (Conversion for CPF: 1 ppm =14.31 mg/m^3; 1 ppm =14.31 mg m^{-3}; X ppm= 1.31 mg m^{-3}; x= 0.09 ppm
f- BMD performed using U.S.EPA Benchmark Dose Software version 2.6; Hill model, 95th percent confidence limit (RBC AChE inhibition analysis in rat at 48 hours).
g- Human volunteers treated at 0.5, 1.0 and 2.0 mg/kg CPF
Abbreviations: c.o. = corn oil
*-Acceptable according to FIFRA Guidelines
"—" = No NOEL

II.D. Subchronic Toxicity

A number of acceptable Health Effects Test guideline subchronic studies are available for CPF as shown below in Table 8 and Table 9. 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 8 (detailed in: HHAB Summary of Toxicology Data; APPENDIX 1).

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

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[a]
Oral					
Rat F-344 M/F	Diet 28 d	↓ plasma ChE	0.05	0.1	1*
Rat SD M/F	Diet 2-Generation Repro	↓ plasma and RBC AChE	0.1	1.0	2*
Rat F-344 M/F	Diet 13 Weeks Neurotoxicity	↓ plasma and RBC AChE	0.1	1.0	3*
Rat Long-Evans F	Gavage c.o. 4 weeks	↓ plasma, RBC and brain ChE	--	1.0	4*
Rat SD F	Diet 28 d	↓ RBC and brain AChE	--	0.4	5*
Rat Wistar M	Gavage c.o. 90 days	↓ spinal cord, brain, plasma ChE	-- 3.26	AChE 1.25 8.15 (HDT)	6
Beagle Dog M/F	Diet 6 weeks	↓RBC AChE	--	0.5	7
Dermal					
Rat F-344 M	21d, 6hr/d, 5d/wk	No effects	--	No LOEL >5	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. Wang et al. (2014); 7. Marable et al. (2001); 8. Calhoun and Johnson (1988)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 HHAB based on FIFRA guidelines
Abbreviations: AChE: cholinesterase; RBC: red blood cell
"—" = No NOEL
a- 1. Marty et al. (2012), Marty and Andrus (2010); 2. Carr et al. (2011); 3. Carr et al. (2013); 4. Carr et al. (2014); 5. Moser et al. (2006); 6. Betancourt and Carr (2004); 7. Timchalk et al. (2006); 8. Richardson and Chambers (2005); 9. Guo-Ross et al. (2007); 10. Zheng et al. (2000); 11. Song et al. (1997); 12. Dam et al. (2000); 13. Mattsson et al. (2000) ; 14. Ouellette et al. (1983); 15. Rubin et al. (1987a); 16. Hoberman (1998); 17. Maurissen et al. (2000); 18. Deacon et al. (1979); 19. Rubin et al. (1987b); 20. Qiao et al. (2002); 21. Speed et al. (2012); 22. Nolan et al. (1984); 23. Kisicki et al. (1999); 24. Calhoun and Johnson (1988); 25. Nolan et al. (1982) and Griffin et al. (1999); 26. Hotchkiss et al. (2010); 27. Hotchkiss et al. (2013); 28. Landry et al. (1986)
b- Milk and corn oil results were the same for males and females except brain AChE with milk: NOEL: 2.0 M and 0.5 F
c- Results were the same with diet and gavage
d- Single exposure
e- Conversion to mg/kg/d: 3.7 mg/m^3 x 0.96 mg/m^3 (breathing rate in rat) x 0.25 (treatment: 6 hr/24 hr) = 0.89 mg/kg (Conversion for CPF: 1 ppm =14.31 mg/m^3; 1 ppm =14.31 mg m^{-3}; X ppm= 1.31 mg m^{-3}; x= 0.09 ppm
f- BMD performed using U.S.EPA Benchmark Dose Software version 2.6; Hill model, 95th percent confidence limit (RBC AChE inhibition analysis in rat at 48 hours).
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II.D. Subchronic Toxicity

A number of acceptable Health Effects Test guideline subchronic studies are available for CPF as shown below in Table 9 and Table 10. Table 9 focuses on NOELs and LOELs for plasma, RBC and brain ChE inhibition in rats, mice and dogs after oral, dermal or inhalation exposure. Table 10 reports subchronic overt (non-ChE) effects in some of the same studies described in Table 9 (detailed in: HHAB Summary of Toxicology Data; APPENDIX 1).

Table 9. AChE Inhibition with Subchronic Exposure to Chlorpyrifos and the Respective NOELs and LOELs

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[a]
Oral					
Rat F-344 M/F	Diet 28 d	↓ plasma ChE	0.05	0.1	1*
Rat SD M/F	Diet 2-Generation Repro	↓ plasma and RBC AChE	0.1	1.0	2*
Rat F-344 M/F	Diet 13 Weeks Neurotoxicity	↓ plasma and RBC AChE	0.1	1.0	3*
Rat Long-Evans F	Gavage c.o. 4 weeks	↓ plasma, RBC and brain ChE	--	1.0	4*
Rat SD F	Diet 28 d	↓ RBC and brain AChE	--	0.4	5*
Rat Wistar M	Gavage c.o. 90 days	↓ spinal cord, brain, plasma ChE	-- 3.26	AChE 1.25 8.15 (HDT)	6
Beagle Dog M/F	Diet 6 weeks	↓RBC AChE	--	0.5	7
Dermal					
Rat F-344 M	21d, 6hr/d, 5d/wk	No effects	--	No LOEL >5	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. Wang et al. (2014); 7. Marable et al. (2001); 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 HHAB based on FIFRA guidelines
Abbreviations: AChE: cholinesterase; RBC: red blood cell
"—" = No NOEL

Table 10. Overt Effects with Subchronic Exposure to Chlorpyrifos and the Respective NOELs and LOELs[a]

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[b]
Oral					
Rat F-344 M/F	Diet 28 d	↓body weights, body weight gains, feed consumption; ↑clinical signs & urinalysis, hematology, clinical chemistry & organ weight effects; ↑fatty vacuolization of the adrenal zona fasciculata	1.0	5.0	1*
Rat SD M/F	Diet 2-Generation Reproduction	Parental:↑ vacuolation in zona fasciculate, altered tinctorial properties in this tissue. Pup: ↓pup weights & pup survival	Parent/Pup: 1.0	Parent/Pup: 5.0	2*
Rat F-344 M/F	Diet 13 Week Neurotoxici	↑ clinical signs, ↑FOB, motor activity effects	1.0	5.0	3*
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	4*
Rat SD F	Diet 28 d	↓absolute & relative spleen & thymus weights; ↑anti-SRBC assay effects	0.4	2.0	5*
Dermal					
Rat F-344 M/F	21 day dermal	No overt effects	5	LOEL>5	6
Inhalation					
Rat -344 M/F	Aerosol, Nose-only; 6 hr/d, 5 d/wk, 13 wk	No overt effects	--	>0.286 mg/m^3	7

a- No subchronic inhalation studies with reported overt effects.
b- 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. 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 HHAB based on FIFRA guidelines
"—" = No NOEL

II.E. Chronic Toxicity/Carcinogenicity

A number of acceptable Health Effects Test guideline chronic studies submitted by the registrant are available for CPF as shown below. Table 11 focuses on NOELs and LOELs plasma, RBC and brain AChE in rats, mice and dogs after oral, dermal or inhalation exposure. Table 12 reports subchronic overt (non-AChE) effects in some of the same studies described in Table 11. There was no significant increase in tumors with any of these long-term studies. These studies are more fully described in the HHAB Summary of Toxicology Data (APPENDIX 1).

Table 11. ChE Inhibition with Chronic Exposure to Chlorpyrifos and the Respective NOELs and LOELs[a]

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[b]
Oral					
Rat F-344 M/F	Diet 2 yr	↓ plasma ChE	**0.05**	0.1	1*
Rat F-344M/F	Diet 2 yr	↓ plasma, RBC and brain ChE	**0.01**	0.1	2*
Dog Beagle M/F	Diet 2 yr	↓ plasma (0.03), RBC (1.0) and brain ChE (0.03)	**0.03**	0.1	3*
Mouse CD-1	Diet 79 wks	↓ plasma, RBC and brain ChE	--	0.78	4*

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

Table 12. Overt Effects with Chronic Exposure to Chlorpyrifos and the Respective NOELs and LOELs[a]

Species	Exposure	Effects	NOEL mg/kg/d	LOEL mg/kg/d	Ref[b]
Oral					
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 (1988a); 2. Crown (1990); 3. McCollister et al. (1971); 4. Gur (1992)
*The study was acceptable to HHAB based on FIFRA guidelines
"—" = No NOEL

II.F. Genotoxicity

Several genotoxicity studies of this active ingredient were submitted by the registrant(s). CPF is not mutagenic in bacteria (Bruce and Zempel 1986; Simmon et al. 1977) or mammalian cells (Mendrala 1985), but did cause slight genetic alterations in yeast (Simmon et al. 1977). Chlorpyrifos did not result in DNA damage in human embryo fibroblasts or rat primary hepatocytes in vitro (Mendrala and Dryzga 1986; Simmon et al. 1977), was not clastogenic in the mouse micronucleus test *in vivo* (McClintock and Gollapudi 1989) and failed to induce unscheduled DNA synthesis in isolated rat hepatocytes (Mendrala 1985). However, studies performed with CPF, using the comet assay (Mehta et al. 2008; Rahman et al. 2002), showed DNA damage. Mehta et al. (2008) treated male Wistar rats with CPF for 1-3 days at 50 or 100 mg/kg/d or for 90 days at 1.12 or 2.24 mg/kg/d. Results showed increased DNA damage in liver and brain at all doses tested in all dosing regimens. Rahman et al. (2002) tested CPR for the ability to induce *in vivo* genotoxic effect in leucocytes of Swiss albino mice using the single cell gel electrophoresis assay or comet assay. The mice were gavaged with CPF (0.28 to 8.96 mg/kg) body weight and whole blood leukocytes were examined at 24, 48, 72 and 96 h. A dose-related increase in mean comet tail length indicating DNA damage was observed at 24h post-treatment ($P<0.05$) with CPF in comparison to control. By 96 h post-treatment the mean comet tail length reached control levels indicating repair of the damaged DNA.

II.G. Reproductive Toxicity

CPF, (98.5% pure) was fed in the diet to Sprague-Dawley rats from premating through F2 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/day. Treatment began approximately 10 and 12 weeks prior to breeding for the F0 and F1 adults, respectively. The cholinesterase inhibition (see **Table 7**) NOEL was 0.1 mg/kg/day (↓plasma and RBC AChE 1.0 and 5.0 mg/kg/day). The parental NOEL was 1.0 mg/kg/day (↑degree of vacuolation in zona fasciculata, especially in males; altered tinctorial properties in this tissue in females). The reproductive NOEL was 1.0 mg/kg/day (slightly reduced pup weights and slightly reduced pup survival at 5.0 mg/kg/day). There were no clinical signs specifically indicating cholinesterase inhibition. The reproductive findings at 5 mg/kg/day 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 which were otherwise normal.

II.H. Developmental Toxicity

Table 13 has acceptable Health Effects Test guideline CPF studies submitted by the registrant as well as open literature studies. All studies are detailed in the HHAB Summary of Toxicology Data (APPENDIX 1) as well as in the U.S.EPA risk assessment documents (U.S. EPA 2007, 2011a, 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 (Table 13).

Table 13. Developmental Effects of CPF and the Respective NOELs and LOELs

Species	Exposure	Effects	NOEL mg/kg/day	LOEL mg/kg/day	Ref[a]
Oral Gavage Treatment to Pups/Neonates					
Rat SD M/F	Gavage c.o. PND 10-16	↓Forebrain, Medulla-Pons and Plasma ChE; ↓hydrolysis of 2-AG and AEA	-- --	1.0 AChE: 1.0	1
Rat SD M/F	Gavage c.o. PND 10-16	↓Forebrain, medulla-pons & plasma ChE, FAAH &MAGL; ↑AEA and 2-AG; With the lowest dosage, peak inhibition of FAAH (52%) is greater than that of BrChE (24%) and that level of FAAH inhibition is sufficient to induce a persistent pattern of elevated AEA.	-- --	1.0 AChE: 1.0	2
Rat SD M/F	Gavage c.o. PND 10-16	↓plasma ChE and CES was present at both 4 and 12 h; No significant inhibition of forebrain AChE or MAGL activities; No significant change in 2-AG at either time point; ↓FAAH activity at 4 & 12h resulting in a significant accumulation of AEA. This demonstrates that developmental CPF exposure at a level that does not inhibit brain AChE can alter components of endocannabinoid signaling.	-- --	0.5 Plasma ChE: 0.5	3
Rat SD M/F	Gavage c.o. PND 10-16	↑Open field effects, elevated plus maze, chasing crawling over/under, play fighting, playing	--	0.5	4
Rat SD M/F	Gavage c.o. PND 10-16	↑time of emergence into illuminated area; ↑DOPAC; ↑HVA	--	0.5	5
Rat SD M/F	Gavage c.o. or milk[d] PND 11	↓RBC, Plasma & BrChE	F: plasma, RBC, Brain 0.5[b]	2	6[c]
Rat SD M/F	Gavage c.o. PND 11-21	↓RBC, Plasma & BrChE	M &F: plasma: 0.05; RBC: 0.1; brain: 0.5	1	6[c]
Oral Gavage Treatment to Dams During Gestation (including DNT)					
Rat F-344 F	Gavage GD 6-15 Cottonseed oil	Dam: Cholinergic signs, clinical signs, decreased body weight gain, enlarged adrenals; ↓ plasma and RBC AChE Fetus: No effects	Dam: 3.0 Fetus: 15 (HDT) ChE Dam: 0.1	Dam: 15 Fetus: No LOEL AChE Dam: 3	7*
Rat CD F	Gavage GD 6-15 Cottonseed oil	Dam: Tremors, ↓ food consumption; ↓body weight; ↓ plasma ChE Fetus: ↑post-implantation loss	Dam/Fetus: 2.5 ChE Dam: --	Dam/Fetus: 15 AChE Dam: 0.5	8*
Mice CF-1 F	Gavage GD 6-15 Cottonseed oil	Dam: Cholinergic signs, ↓ food and water consumption, ↓body weight gain; ↓ plasma and RBC AChE Fetus: ↓live fetuses; ↓body weight; ↓crown-rump length; ↑delayed ossification in skull & sternabrae	Dam: 1.0 Fetus: 10 ChE Dam: --	Dam: 10 Fetus: 25 AChE Dam: 0.1	9*
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 ChE --	Dam/Fetus: 140 AChE 1.0	10*
Dermal Treatment Pups and Adults					
Mice Balb/c M Adult (150 d) Pup (18 d)	4 hr/d, 2 weeks: 1 dose level administered on the tail	Adult: ↓ Plasma ChE; dissolution of Nissl granules; ↑GPAF[f] Pup: ↓ Plasma ChE; pyknosis in Purkinje neurons in cerebellum	Pup/Adult --	Pup/Adult: 101	11

References: 1. Carr et al. (2011); 2. Carr et al. (2013); 3. Carr et al. (2014); 4. Carr et al. (2015); 5. Mohammed et al. (2015); 6. Marty and Andrus (2010); 7. Ouellette et al (1983); 8. Rubin et al. (1987); 9. Deacon et al. (1979); 10. Rubin et al. (1987); 11. Krishnan et al. (2012)
b- milk & corn oil same results m & f except brain AChE with milk 2.0 M and 0.5 F
c- results same with diet and gavage
d- single exposure
e-Only looked at ChE inhibition
f-GPAF Glial fibrillary acidic protein is the principal intermediate filament protein found predominantly in mature astrocytes of the central nervous system. It is critical in the regulation of astrocyte motility. Astrocytes become reactive following variable injury to the CNS. The cells respond in a distinctive manner termed reactive gliosis rapidly increasing the expression of GFAP (Pekny et al., 1999).
*The study was acceptable to HHAB based on FIFRA guidelines
"—" = No NOEL

II.I. Neurobehavioral Developmental Neurotoxicity

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

Table 14. Chlorpyrifos Treatment Postnatally and the Neurobehavioral Effects Measured Post-weaning

Species	Dosing Period	ChE Inhibition	Domain Affected	Age of Testing	NOEL	LOEL	Ref
Oral Gavage or Subcutaneous Injection to Pups/Neonates							
Rat SD M/F	Gavage c.o. PND 1-21	PND20 (time after dose not given) ↓14-53% hippocampal ChE all doses M/F	↓ Cognition	PND 36-60	NOEL <1.0	1.0	1
Rat SD M/F	Gavage c.o. PND 1-21 every other day	PND 25 & 30: ↓14-26% brain ChE at all doses	↓ Motor activity	PND 25, 30	3.0	6.0	2.
Rat SD M/F	Gavage c.o. PND 10-16	Not tested	↑Open field effects, elevated plus maze, chasing crawling over/ under, play fighting, playing	PND 25	NOEL <0.5	0.5	3
Rat SD M/F	Gavage c.o. PND 10-16	Not tested	↑time of emergence into illuminated area; ↑DOPAC; ↑HVA	PND 25	NOEL <0.5	0.5	4
Rat Long-Evans M/F	s.c. Peanut oil PND 11, 15	No ↓brain ChE PND7 (3 hr after dose), 8, 16, or 28	↓ Cognition	PND 24-28	<0.3	0.3	5
Mouse CD-1	s.c. Peanut Oil PND 11-14	Not tested	↑Social behavior & maternal interaction all intervals (dam & pup)	PND 40-45; LD 1-7 & 7	One dose; NA	3.0	6
Rat SD M/F	s.c. DMSO PND 1-4	PND 1 only day tested: ↓60% M brain; ~20% F	↓ Motor activity	PND 21, 30	One dose; NA	1.0	7
Rat SD M/F	s.c. DMSO PND 1-4	Not tested	↓ Cognition;↓ anxiety; ↑motor activity	PND 52-53 & 64+	One dose; NA	1.0	8
Oral Gavage or Subcutaneous Injection to Dams During Gestation							
Rat SD F	Gavage c.o. GD 6-LD 11	Dam Only: ↓Brain (≥1.0), RBC, Plasma ChE (≥0.3)	↓ motor activity ↓ neuromotor function	PND 12-71	Dam: 1.0 Pup: 0.3 AChE Dam: 0.3	Dam: 5.0 Pup: 1.0 AChE Dam: 1.0	9*
Rat SD F	Gavage c.o. GD 6-LD 10	Dam: ↓RBC, Plasma & BrChE	↓ neuromotor function	PND 11-70	Pup: 5 AChE Dam: NOEL --	Pup: 5 AChE Dam: 0.3	10
Mouse CD-1 F	Gavage peanut oil GD 14-17	Not tested	↑Anxiety, emotion & social behavior	PND 90; Adult F after mating on post-partum	Only 1 dose tested	6.0	11

Table 14. Chlorpyrifos Treatment Postnatally and the Neurobehavioral Effects Measured Post-weaning

Species	Dosing Period	ChE Inhibition	Domain Affected	Age of Testing	NOEL	LOEL	Ref
				day 8			
Mouse CD-1	s.c. Peanut Oil GD 15-18 & PND 11-14	Not tested	No effect on social behavior (only F pups tested)	PND 120	3.0	>3.0 HDT	12
Mouse CD	s.c. peanut oil GD 15-18 & PND 1-14	Not tested	↑ motor activity, ↓ anxiety & emotion, ↑social behavior	PND 90, 75-80, 120	3.0	6.0	13
Rat SD	s.c. DMSO GD 9-12	Not tested	↑Motor activity ↓Cognition	PND 28-91	Dam: 1.0 Pup NOEL --	Dam: 5.0 Pup: 1.0	14
Mouse HS/lbg F	s.c. DMSO GD 9-18	Not tested	↓Cognition	Pups PND 75	NOEL --	1.0	15
Mouse HS/lbg F	s.c. DMSO GD 9-18	Not tested	↓Cognition	PND 80	Only 1 dose tested	3.0	16
Mouse Swiss Webster F	s.c. DMSO GD 17-20	Not tested	↓Cognition	PND 60-81	NOEL --	1.0	17
Rat SD	s.c. injection DMSO GD 17–20	Not tested	↑Motor activity ↓Cognition	PND 28-42, 56-91	NOEL --	1.0	18.
Mouse CD-1	s.c. DMSO PND 1-4	PND 4 ↓20, 23% brain AChE 1 hr post-dose	↓Social behavior; ↑motor activity	PND 25, 35-38, 38, 45, 60	NOEL --	1.0	19
Mouse CD-1	s.c. DMSO GD 15-18 & PND 11-14	↓Plasma ChE (24 hr after final dose; both doses)	↑Social behavior & maternal interaction (dam & pup); ↑motor activity; ↓anxiety	PND 70, 75-80, 90, 120	NOEL --	1.0	13
Dermal Treatment to Dams During Gestation							
Rat SD	Dermal (70% ETOH) GD4-20	↑0-30% brain AChE PND90, F	↓Neuromotor function	PND 90	One dose; NA	1.0	20

a-Parameters include neuropathology, brain weights, morphometrics, motor activity, body temperature, auditory startle response, delayed spatial alternation
References: 1. Johnson et al. (2009); 2. Carr et al. (2001); 3. Carr et al. (2013); 4. Mohammed et al. (2015); 5. Jett et al. (2001); 6.Venerosi et al. (2008); 7. Dam et al. (2000); 8. Aldridge et al. (2005a); Aldridge et al. (2005c); 9. Hoberman (1998); 10. Maurissen et al. (2000); 11. Venerosi et al. (2010); 12. Venerosi et al. (2006); 13. Ricceri et al. (2006); 14. Icenogle et al. (2004); 15. Billauer-Haimovitch et al. (2009); 16. Turgeman et al. (2011); 17. Haviland et al. (2010); 18.Levin et al. (2002); 19. Ricceri et al. (2003); 20. Abou-Donia et al. (2006)
"—" = No NOEL

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/day for 28 days (Boverhof et al. 2010). Another 10 females were dosed by intraperitioneal (i.p.) injection with 20 mg/kg/day of cyclophosphamid 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/day and the immunology NOEL was 0.4 mg/kg/d.

II.K. Epidemiological Studies Related to Neurodevelopmental Effects

II.K.1. Children's Health Studies

Several studies were performed evaluating effects of organophosphates (OPs) on development in children after CPF exposure to mothers during pregnancy. The summaries of the main epidemiology study are described below and reviewed elsewhere (Reiss et al. 2015) (Mink et al. 2012). The reviews concern results of exposure to numerous organophosphate pesticides and not exclusively CPF. In the study populations (each with a different exposure scenario), women were recruited and evaluated during pregnancy and the effects from CPF were assessed in their newborns and young children. Columbia University's Mother's and Newborn Cohort Study (CCCEH Cohort: Columbia Study) was one-such well-conducted and ongoing study that reported findings primarily focused on the effects of CPF exposure. The above study was chosen to be the focus of discussions related to maternal and fetal exposure and neurobehavioral and developmental effects in children.

II.K.1.a. The Columbia University's Mother's and Newborn Cohort (CCCEH Cohort "Columbia Study") (Horton et al. 2012; Lovasi et al. 2011; Perera et al. 2003; Rauh et al. 2011; Rauh et al. 2006; Rauh et al. 2012; Reiss et al. 2015; Whyatt et al. 2009; Whyatt et al. 2007; Whyatt et al. 2004)

Pregnant African-American and Dominican women (18-35 years of age;) known to be exposed to CPF were recruited in early pregnancy (≤20 weeks; 1998-2004) to evaluate the effects on development in their children through the age of 9 years (725 mother-child pairs enrolled, 70% participation as of 2002; 83% retention 3-year follow-up, 82% retention at 7-year follow-up) (Rauh et al. 2012). The recruitment years overlap the voluntary cancellation of CPF for residential use (2000-2001). 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 parent CPF in fetal cord blood, in maternal and fetal urine (TCPy) and meconium (within 2 days of delivery) and in residential/environmental personal air via monitors during the third trimester of pregnancy. Participants responded to questionnaires in their homes during the last trimester of pregnancy and then yearly. The birth outcomes, delivery outcomes and related medical information were also obtained for each participant. Cord blood and maternal blood levels were considered to be almost 1:1 ratio (>80% correlation). Subsequently children were measured on the Bayley Scales of Infant Development (Mental Development Index and Psychomotor Development Index and Child Behavior Checklist), and Wechsler Intelligence Scale for Children (Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory Index, and Processing Speed Index); Brain morphology assessed using high-resolution, T1-weighted magnetic resonance imaging at 5.9 – 11.2 years (summarized from Reiss et al., 2015).

Early results showed an inverse relationship between CPF in fetal cord blood and birth weight and length outcomes in neonates in the CCCEH cohort (Perera et al. 2003). Using the parent CPF as a biomarker, Whyatt et al. (2004) then confirmed an association between *in utero* exposure to CPF (> 6.17 pg CPF/g cord blood plasma) and reduced birth weight and birth length, increased risk of small size for gestational age. The limit of detection (LOD) for CPF in blood samples was 0.5–1 pg/g plasma (Whyatt et al. 2009). At age 3, the same cohort of children with exposure to CPF at 6.17 pg CPF/g showed "increased risk of mental and motor delay (< 80 points) and 3.5-6-point adjusted mean decrements on the 3-year Bayley Scales of Infant Development and evidence of increased problems related to attention, attention deficit hyperactivity disorder, and pervasive developmental disorder as measured by the Child Behavior

Checklist at 2–3 years" (Rauh et al. 2006). The same cohort of children were again examined at age 7 years to estimate the long term effects prenatal CPF exposure on neurodevelopment (Rauh et al. 2011). Both working memory and Full-Scale intelligence quotient (IQ) were decreased by 2.8% and 1.4%, respectively, for each standard deviation (± 4.61 pg/g) increase in CPF exposure.

Rauh et al. (2011) next performed magnetic resonance imaging studies on 40 children (ages 5.9-11.2 years old) from the CCCEH birth cohort to see if CPF exposure in utero affected brain morphology. Children exposed at high concentrations (n = 20; upper tertile of CPF concentrations in umbilical cord blood) were compared to those with low-exposure (n = 20) for cortical surface features. Numerous morphological differences were reported in the children with high CPF exposure (enlarged superior temporal, posterior middle temporal, and inferior postcentral gyri bilaterally, and enlarged superior frontal gyrus, gyrus rectus, cuneus, and precuneus along the mesial wall of the right hemisphere). High exposure children had frontal and parietal cortical thinning, and an inverse dose–response relationship between CPF and cortical thickness. There were no sex differences among high-exposure children in areas of the brain where they would be expected (enlargement of the right inferior parietal lobule, supramarginal gyrus, and bilateral superior and middle temporal gyri in females (31, 32), and enlargement of the left mesial surface of the superior frontal gyrus in males) (Cahill 2006; Harasty et al. 1997; Nopoulos et al. 2000). Instead there was a reversal of sex differences in the high exposure group similar to those reported in animal models where early exposure reverses normal sex differences in learning, memory, and emotional behaviors (Aldridge et al. 2005a; Aldridge et al. 2004; Levin et al. 2001). These effects were not seen in low-exposure children but there was a significant exposure × IQ interaction which was derived from CPF disruption of normal IQ associations with normal surface measures.

Some of the children in the CCCEH birth cohort received neuropsychological assessments to identify potential long-term effects of prenatal CPF exposure on neuro-motor development (Rauh et al. 2015). For this study the children were divided into the high exposure (>6.17 pg/g) or lower exposure (≤6.17 pg/g), as described in Rauh et al (2006; CPF concentration range: 0.25 - 63 pg/g). Possible motor effects at low to moderate levels of exposure have not been evaluated. At a mean age of 11 years (mean = 10.9 ± 0.85 years, range = 9.0 – 13.9), children were asked by a senior neurologist specializing in movement disorders (blind to CPF exposure level) to draw Archimedes spirals. Compared to all other children, those with prenatal CPF exposure in the upper quartile (>6.17 pg/g; n=43) had mild or mild to moderate tremor (>1 rating) in either arm (p=0.03), both arms (p=0.02), the dominant arm (p=0.01), and the non-dominant arm (p=0.055) after adjustment for sex, age at testing, ethnicity, and medication. Therefore, children assessed at age 9-13 after exposure *in utero* to CPF at >6.17 pg/g (high exposure) were significantly more likely to show mild or mild to moderate tremor in one or both arms. The proportion of the children with high exposure had mild or mild to moderate tremor (16.3-39.5%), depending on the arm, compared to low exposure values of 6.1-22.8%. The study authors did not speculate as to what may be the basis of the tremor but definite nerve dysfunction, including CNS-generated tremors (i.e., tremors that arise from brain dysfunction) and PNS-generated tremors (i.e., tremors that arise from peripheral nerve dysfunction) (Deuschl et al. 1998). There was also increased incidences of attention deficit disorder and attention-deficit/hyperactivity disorder (ADHD) (Rauh et al. 2006).These data strongly indicate that exposure to CPF *in utero* is associated with tremor years later in childhood, which means that CPF is toxic to the CNS and the PNS.

The generalized effect on white matter integrity (enlargement) and reduced cortical thickness in scattered areas across the brain surface in children exposed to higher levels of CPF was confirmed. Some reversal of usual female vs. male differences in sexually dimorphic brain regions (e.g. parietal lobe size)

was seen in high exposure children (high CPF exposed ≥4.39 pg/g; low CPF exposed <4.39 pg/g). Morphologic changes appeared to be related to lower IQs in these children and the results were found to "support the contention that exposure to CPF, even for some in the low CPF exposure group, is related to general cognitive deficits." In addition, the results provided "convergent evidence" with the findings of a reduced thickness of the parietal cortex in rat offspring in the DNT Health Effects Test Guideline study (Hoberman 1998) submitted by Dow AgSciences. Another conclusion was that "the results of this study suggest that one might expect that the most common effects of CPF exposure would be similar to the most common effects associated with a range of developmental brain insults, effects such as attention deficits, learning disabilities and deficits in social development."

II.K.1.b. CPF Doses to Women of the Columbia Cohort and Neurodevelopmental Impairment—A Risk Projection Reflecting Inputs from Different Sources of Information

Neurodevelopmental effects of CPF reported in the Columbia Cohort study indicated that there was a relationship between decrements in working memory in children at age 7 and CPF in fetal cord blood levels at birth (Rauh et al. 2011). Working memory, an executive function is a component of IQ and is necessary for other cognitive processes. It was reported to be the most affected aspect of IQ in the CPF exposed children (Rauh et al. 2011). A normal population has a working memory with an "index of function defined as having a mean of 100 and a standard deviation of 15." In order to use these data for regulatory purposes, Hattis (2015) developed a model to translate measured CPF levels in fetal and maternal blood into external exposures at low doses. This model allows for an analysis of the risk of exposure to CPF *in utero* on neurodevelopment in children. Human data from four sources (Kisicki et al. 1999; Nolan et al. 1984; Rauh et al. 2011; Smith et al. 2011) were utilized in a recalibrated version of the PBPK-PD model of Timchalk (2008). The recalibrated model used *in vitro* human metabolism data (Smith et al. 2011) and incorporated "parameter uncertainties" to derive "low-dose dosimetry translation factors." Although the exact duration of exposure for the women in the Columbia Cohort was not known, the model was based on steady-state levels of CPF (achieved after 400 hrs). A dose-response analysis was modeled to estimate CPF exposures that were received by women of the Columbia cohort during pregnancy (measured in maternal blood: LOD 0.5 – 1.0 pg/g blood plasma) and the loss in working memory in their 7 year old children after exposure *in utero* (based on measured in fetal cord blood). The modeled low-dose dosimetry translation factors, the blood measurements and the effects in children could be "considered as directly observed rather than estimated from a high-dose to low dose projection."

Bounding values for inhalation exposures were estimated from a lognormal distribution defined by the ratios of air intake to maternal blood concentrations as the lower 1% bound and the ratio of urinary TCP excretion to maternal blood concentration as the upper 99% bound (Hattis 2015). The central estimate is the geometric mean of distributions; the lower and upper estimates are the 2.5% and the 97.5% confidence limits, respectively. Bounding estimates assume the bulk of the CPF exposure to the cohort was via the inhalation route. A central estimate of the inhalation exposure required to attain a blood concentration of 6.17pg/g is calculated as follows: (6.17pg/g) x (27.6 [ng CPF absorbed/kg body weight-d]) = 170 ng/kg/d; lower and upper-bound inhalation estimates are 130 and 191 ng/kg/d, respectively. Oral exposure (228 ng/kg/d) is estimated by obtaining the central estimate dosimetry ratio of 33.7 (geometric mean of [ng CPF absorbed/kg body weight-day]/pg CPF/g maternal blood plasma) and multiplying it by 6.17 pg/g (Hattis 2015; Rauh et al. 2006).

Therefore the PoD for steady-state oral exposure (most likely the current route for human) was approximately 228 ng/kg/d (range =141-369 ng/kg/d) for pregnant women (Hattis 2012; Hattis 2015) based on a low CPF exposure level (<6.17 pg/g) (Table 15). Oral exposures need to be approximately 20%

greater than inhalation exposures to result in a comparable effect on blood levels based on a comparison of central estimates (geometric means). Accordingly, median, lower and upper-bound estimates of ingestion exposure that result in a blood concentration of 6.17pg/g, are 208, 117 or 308 ng/kg/d, respectively. This information might be useful when adapting oral PoDs to inhalation exposures. Though these data are preliminary at this time, they provide supportive evidence that UFs are needed for regulatory end-points based on neurodevelopmental and neurobehavioral effects in humans.

Table 15. Bounding estimates of the CPF maternal dose during pregnancy (ng/kg/d) corresponding to 1 pg/g CPF in umbilical cord blood of newborns at delivery[a]

Lower estimate dosimetry ratio[b]		Central estimate dosimetry ratio[b]		Upper-bound estimate dosimetry ratio[b]	
Inhalation	**Ingestion**	**Inhalation**	**Ingestion**	**Inhalation**	**Ingestion**
21.1	19.0	27.6	33.7	31.0	49.9

a-It is assumed that much of the CPF absorbed via the oral route is subject to first pass hepatic metabolism.
b-Data in Hattis (2015) and Rauh et al. (2006)

II.K.1.d. UC Berkeley's the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) Cohort (Bouchard et al. 2011; Eskenazi et al. 2004; Eskenazi et al. 2010; Eskenazi et al. 2007; Harley et al. 2011; Marks et al. 2010; Raanan et al. 2015; Young et al. 2005)

Low-income, primarily Mexican-American or Mexican immigrant female subjects were farm laborers and/or were living with someone employed as a farm laborer in Salinas Valley, CA (Eskenazi et al. 2004). Although this is one of the highest areas of agricultural production in the United States (>500,000 pounds of organophosphate pesticides [OP] are applied annually), CPF was not highly used during the time of the study. This study was designed to evaluate OP exposure in general and was not directed at CPF exposure specifically, however, TCPy (a biomarker of CPF exposure) was assessed. Self-reported questionnaires were provided for participating women. OP metabolites dialkyl phosphate (DAP) and TCPy in maternal urine were evaluated between 5 and 27 weeks gestation; between 18 and 39 weeks and within 1 week of delivery or within 176 days post-partum. Total pesticide metabolite levels were determined for each woman to estimate prenatal pesticide exposure. The levels of BuChE and RBC AChE served to estimate OP exposure; plasma PON1 was also measured. Results suggested that measurement of urinary TCP did not reliably allow quantitative estimation of the children's everyday environmental exposures specifically to CPF but is a reliable general biomarker for OP exposure (Morgan et al. 2011). Other data, however, suggest that TCPy is a specific biomarker to CPF metabolism (Eaton et al. 2008; Timchalk et al. 2007).

A study by Furlong (2007) used transgenic mice that expressed human $PON1_{192}$ phenotypes to examine their function in CPF detoxification. The $PON1_{192}$ protein polymorphism among humans occurs as an amino acid substitution (Gln/Arg) at position 192 of 354-amino acid. Human PON1 DNA has been sequenced and there are two main amino acid polymorphisms "L55M and $Q_{192}R$." There are also three PON1192 functional phenotypes (Q/Q;Q/R;R/R). The "$Q_{192}R$" polymorphism determined high versus low PON1 activity with $PON1_{R192}$ catalytic efficiency for inactivating CPF-oxon >> $PON1_{Q192}$. Variability in PON1 catalytic efficiency of (for example) CPF-oxon hydrolysis based on the level of PON1 protein, may vary by 15-fold among humans that have the same $PON1_{192}$ genotype but different phenotype.

A study by Diepgen and Geldmacher-von Mallinkrodt (1986) helps illustrate both the ethnic diversity of PON1 metabolism and what was later shown to be a trimodal phenotypic distribution of the PON192 allotypes (Q/Q;Q/R;R/R) (Figure 6).

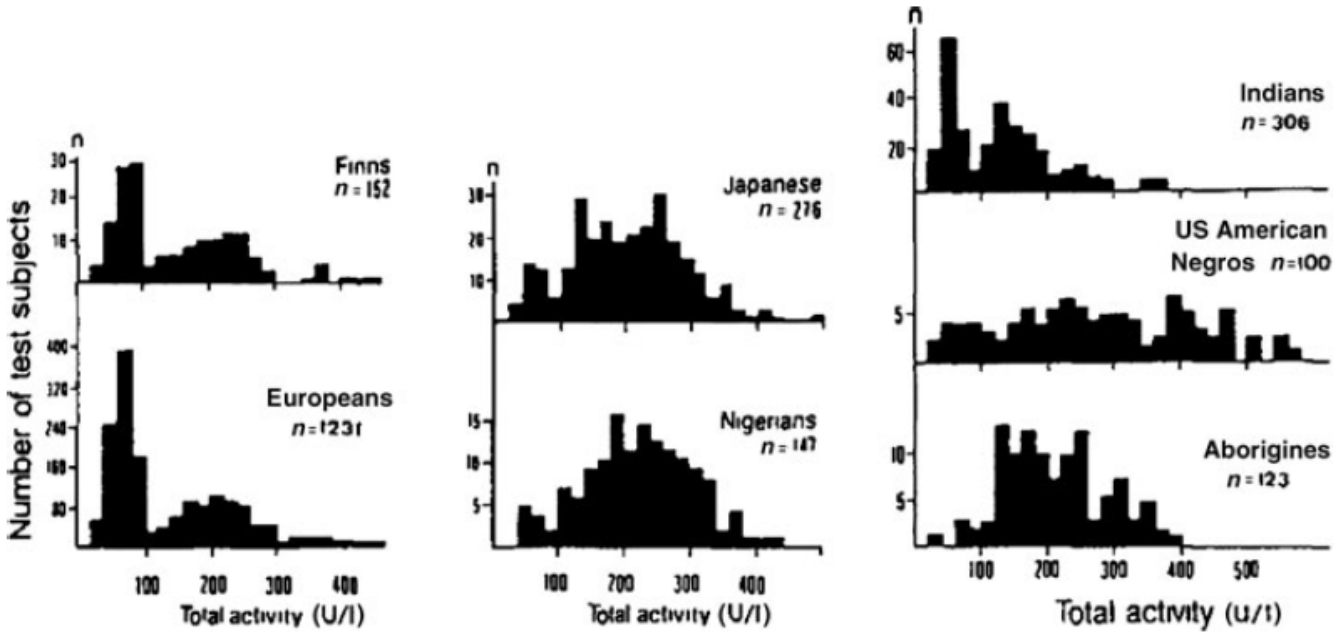


Figure 6. Illustration of PON1 activity diversity among ethnic groups (Diepgen and Geldmacher-von Mallinckrodt 1986)

Data from the CHAMACOS cohort (Salinas Valley, CA) of pregnant Latina women and their newborns was evaluated by Furlong et al. (2006) to evaluate their PON1 activity as a predictor of sensitivity to OP toxicity. Although CPF was not specifically studied, PON1 is a major deactivator of CPF-oxon and therefore genetic variability in levels of this enzyme can affect toxicity. The PON1 activity was compared with the $PON1_{192}$ genotype (and phenotype QQ, QR and RR) status of the Latina mothers (n=130) and their newborns by measurement of arylesterase (AREase) activity. The difference in PON1 (AREase) activity among mothers was 14-fold and was 26-fold in newborns. In addition, the PON1 levels in the children were 4-fold lower than that of their mothers. Based on their findings, the predicted range of variability (sensitivity) to CPF-oxon was 164 fold for mothers and children. This indicates that some of the mothers and children would be more vulnerable to CPF-oxon toxicity than others based on their PON1 activities, especially those with low values. The average PON1 levels in neonates were shown to be comparable to those found in transgenic mice expressing human $PON1_{Q192}$ or $PON1_{R192}$ (Furlong 2007). This finding indicates that the transgenic mouse model may be used to predict relative sensitivity of newborns to CPF-oxon.

The mothers (n=359) and children from the CHAMACOS birth cohort discussed above were also examined for effects of OPs (primarily CPF) on respiratory disfunction in children of the mothers who had been exposed during pregnancy (Raanan et al.). Dialkyl phosphate (DAP) metabolites of OP pesticides (non-specific biomarkers for OP exposure) were measured in urine from mothers twice during pregnancy (mean = 13 and 26 weeks gestation) and from children five times during childhood (0.5–5 years). Childhood DAP concentrations were estimated by the area under curve (AUC). The results of this study indicated that children exposed to OPs (e.g., CPF) prenatally, as indicated by the presence of DAP metabolites (particularly DE from CPF) assessed in the 2^{nd}-3^{rd} trimester of pregnancy is associated with increased odds of respiratory symptoms occurring 5-7 years postnatally. Therefore, early-life exposure to OP pesticides, particularly CPF, was associated with respiratory symptoms consistent with childhood athsma.

II.K.1.e. The Mount Sinai Hospital Children's Environmental Health Cohort (Berkowitz et al. 2004; Engel et al. 2007; Engel et al. 2011)

This prospective birth cohort study examined primiparous women who may have been exposed to CPF (and other pesticides) during pregnancy. The mothers attended the Mount Sinai prenatal clinic and two private practices and delivered their infants at Mount Sinai Hospital in New York City (May 1998-July 2001). They were screened and excluded for various potentially confounding birth parameters (initial prenatal visit after 26 weeks of gestation, serious chronic diseases, a serious pregnancy complication that could affect fetal growth and development; risky health behaviors including alcohol consumed greater than two alcoholic beverages per day or illicit drug use; child was born with a congenital malformation or severe prematurity). Evaluation for exposure to pesticides prenatally was via a self-report questionnaire where information about in home pesticide use, presence of pests and other exposure characteristics. Urine from the cohort was obtained in the 3rd trimester and the concentration of both TCPy (indicator of CPF exposure; Berkowitz et al. (2004)), and non-specific measures of OP (dialkyl phosphates, DAPs) (Engel et al. 2007; Engel et al. 2011) and at birth infant cord blood samples were obtained. The metabolites were evaluated (Barr et al. 2005) as well as PON1 enzymatic activity levels and PON1 genotypes. Infant genotyping was also performed to determine prevalence of PON1 variant alleles (phenotypes). Results showed no statistically significant associations of CPF exposure *in utero*, TCPy concentrations with birth length or birth weight. Most of the cord blood CPF values were at or near the limit of detection (LOD) (Barr et al. 2010). There was a significant CPF-related trend in decreased head circumference and $PON1_{192}$ RR genotype in subjects with detectable TCPy. The $PON1_{192}$ RR genotype is associated with decreased detoxification of CPF-oxon and (based on genotype) can indicate CPF detoxification activity.

Engel et al. (2011), utilizing data from the Mount Sinai Children's Health Study examined the relationship between PON1 (biomarker of OP clearance and de-facto exposure), and cognitive development at ages 12 and 24 months and 6–9 years. In this study, third-trimester maternal urine was analyzed for OP metabolites (n = 360). Blood samples were analyzed in pregnant women for PON1 activity and genotype. Subsequently the children received neurodevelopmental assessments at 12 months (n = 200), 24 months (n = 276), and 6–9 (n = 169) years of age. DAP levels were associated with decreased mental development at 12 months in blacks and Hispanics. The associations were greater among children of mothers who carried the PON1 Q_{192}R QR/RR genotype. Children with mothers who had the PON1 Q_{192}R QQ genotype (associated with slow catalytic activity for CPF-oxon) and increased prenatal total dialkyl- and dimethylphosphate metabolites were shown to have decrements in perceptual reasoning that were observed later in childhood. This association indicates a "monotonic trend consistent with greater decrements with increasing prenatal exposure." Their results support the association of prenatal exposure to OPs with negative effects on cognitive development (perceptual reasoning) and the effects, manifest at 12 months of age, continue throughout childhood. The presence of PON 1 genotypes and phenotypes with slower catalytic activities appear to be indicators of susceptibility to these effects.

II.K.1.f. Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural Pesticides: The CHARGE Study (Shelton et al. 2014)

This study used data from an ongoing case-control study "Childhood Autism Risks from Genetics and Environment (CHARGE) study." Women in the study were exposed to agricultural pesticides (carbamates, OP, organochlorines and pyrethroids) during gestation to agricultural pesticides (Shelton et al. 2014). The CHARGE study has enrolled over 1,600 participants since 2003 whose parents answer extensive questionnaires regarding environmental exposures including their place of residence during pregnancy. Children within California with full autism spectrum disorder (ASD) or developmental delay

(DD) were selected as participants. Exposure was based on the CDPR publically available data source: Pesticide Use Report (PUR). A questionnaire was administered to the mothers that included residential address at 3 months prior to and during pregnancy. The controls were from the general population and were matched to cases. CPF, due to its association with neurodevelopmental disorders in children after exposure *in utero* (Rauh et al. 2012) was evaluated independently. Results showed that pregnant women were exposed to 21 unique compounds (OPs within 1.5 km of the home), the highest of which was CPF (20.7%), followed by acephate (15.4%), and diazinon (14.5%). In addition pyrethroids (esfenvalerate (24%), lamdacyhalothrin (17.3%), permethrin (16.5%), cypermethrin (12.8%), and tau-fluvalinate (10.5%)), carbamates (80% methomyl or carbaryl), and organochlorines (60% dienochlor) were detected. CPF exposure was associated with increased ASD. However this may be difficult to assert because of the numerous pesticide exposures and although one can guess at potential effects by volume of exposure, there are too many compounds to establish a correlative relationship for any individual pesticide. Authors classify "exposure" based on the PUR database which summarizes pesticide uses reported in 1 square mile increments, and such use does not necessarily lead to exposure in individuals living in an area. Although there are limitations in assigning exposures based on the PUR and stated residential addresses, the CHARGE investigators ***assumed*** proximity to pesticide exposure was equated to PUR values. In contrast, in our review we need to maintain the distinction as it is an important one. However, CPF is volatile enough to drift but a person at risk needs to be downwind of the application, not just living in the same neighborhood. The CHARGE Study authors also listed several potential confounders (exposure misclassification, errors in PUR database, hours spent in the home or elsewhere not available, lack of association in time of exposure and effects observed).

II.L. ToxCast/Tox21 Studies

The Toxicity Forecaster (ToxCast^{TM}) program was launched by the U.S.EPA in 2007 as part of the "Toxicity Testing in the 21st Century (Tox21)" Federal program in collaboration with the National Toxicology Program at the National Institute of Environmental Health Sciences, the National Institutes of Health's National Center for Advancing Translational Sciences and the Food and Drug Administration (http://www.epa.gov/chemical-research/toxicity-forecasting; accessed 12-2015). ToxCast was designed to prioritize chemicals based on the results of high-throughput screening (HTS) assays indicating potential disruption of key biological pathways. Chemicals were selected for screening by the U.S.EPA (ToxCast) and the Tox21 collaborators, as well as international programs (OECD) and other stakeholder groups. Currently the multi-phase ToxCast program, with over 700 unique assays and 300 signaling pathways, has evaluated numerous chemicals (~2,000) with established or unknown toxicity, including cosmetics, drugs, pesticides, and environmental contaminants (Tice et al. 2013). The ToxCast data may be used to elucidate biochemical mechanisms as well as common pathways for human disease outcomes. Ultimately a goal of this U.S.EPA program is to use the ToxCast hazard and exposure data predicted by computer modeling to facilitate chemical risk assessments and prioritization.

II.L.1. U.S.EPA ToxCast Assays *In Vitro*

Results were obtained from the seven ToxCast assay platforms that reported active results for CPF and CPF-oxon ("actives"): ACEA Biosciences, Inc. (ACEA), Apredica (APR), Attagene (ATG), Bioseek (BSK), CEETOC (Cyprotex), CellzDirect (CLD), Novascreen (NVS) and Odyssey Thera (OT), the NIH Chemical Genomics Center (NCGC or Tox21) 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 16 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/dashboard2/), 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 16. 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
NCGC (Tox 21)	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 ZF	*Danio rerio* Whole animal[d]	NA	Malformations, neurobehavioral	Developmental Pathways	Visual/ Morphological

a-Primary cultures from Primary human venule endothelial, Primary Human Vascular Smooth Muscle Cells, Primary Human Dermal Fibroblasts, Peripheral blood mononuclear + endothelial
b-BSK tests for: cytokine, cell adhesion, cell cycle, gpcr, growth factor, protease inhibitor, proteases depending on cell types assaye.
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). ZF results are available with the other ToxCast results at: http://actor.epa.gov/dashboard2/

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

Table 17 below shows all assays that were reported as a "hit" or "active" for CPF and indicates the intended target family and assay component endpoint involved (available at: http://actor.epa.gov/dashboard2/ accessed 12/2015). Many are non-specific (e.g., BSK assays) and are associated with various pathways and pathologies (Kleinstreuer et al. 2013; Kleinstreuer et al. 2011) that are not related to CPF toxicity. The "true actives" (assays that are not within the range of cytoxicity: see Figure 8, below) highlighted in Table 17 reveal generalized activities that are not specific to AChE or neurotoxicity. However there were 12 assays that were active for hormone receptors (thyroid, androgen, estrogen) and hormone inhibition (cortisol, progesterone, androgen, testosterone), even in the absence of metabolic activation Table 17. This indicates that CPF can affect endocrine disrupting functions without

being activated to CPF-oxon (assay results not shown), but only at high concentrations that are likely also cytotoxic (see below).

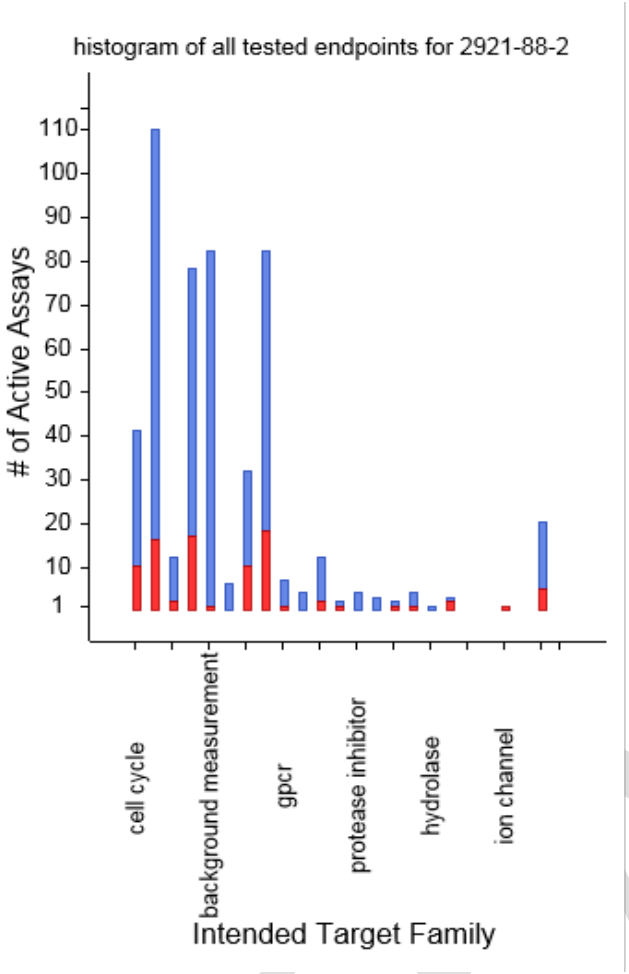
Table 17. ToxCast Assays for Chlorpyrifos

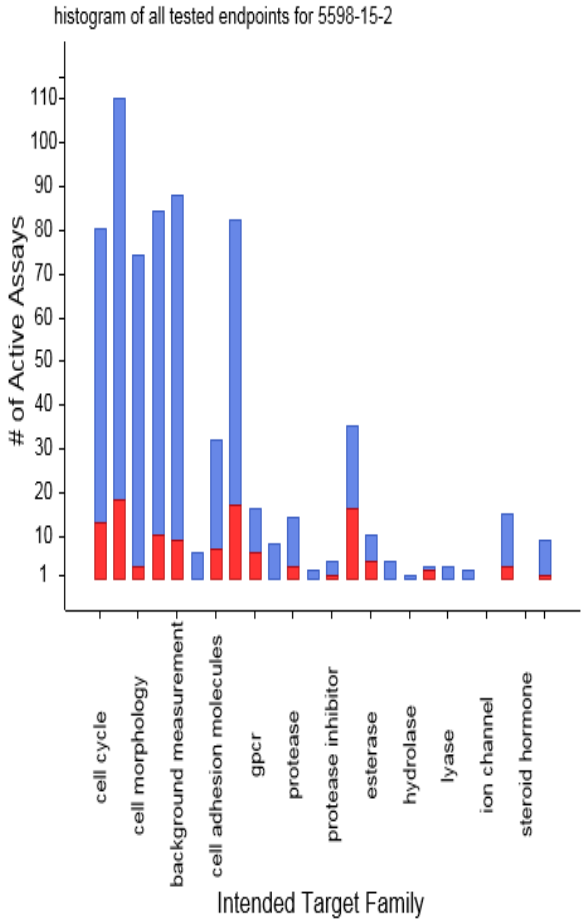
IntendedTarget Family	Assay Component Endpoint Name[a]	AC_{50}	Log AC_{50}	True Active[b,c]
Background Measurement	ATG_CMV_CIS_up	23.84	1.38	--
Cell Adhesion Molecules	BSK_SAg_Eselectin_down	13.10	1.12	+
	BSK_LPS_VCAM1_down	22.22	1.35	--
	BSK_hDFCGF_VCAM1_down	5.78	0.76	+
	BSK_hDFCGF_CollagenIII_down	6.84	0.83	+
	BSK_BE3C_HLADR_down	13.18	1.12	+
	BSK_4H_VCAM1_down	16.03	1.20	--
	BSK_4H_Pselectin_down	33.82	1.53	--
	BSK_3C_VCAM1_down	20.67	1.32	--
	BSK_3C_HLADR_down	16.33	1.21	--
	BSK_3C_Eselectin_down	33.92	1.53	--
Cell Cycle	BSK_SAg_Proliferation_down	27.08	1.43	--
	BSK_SAg_PBMCCytotoxicity_down	26.99	1.43	--
	BSK_hDFCGF_SRB_down	14.32	1.16	+
	BSK_hDFCGF_Proliferation_down	11.53	1.06	+
	BSK_CASM3C_Proliferation_down	14.17	1.15	+
	BSK_4H_SRB_down	15.92	1.20	--
	BSK_3C_SRB_down	15.57	1.19	--
	BSK_3C_Proliferation_down	16.73	1.22	--
	ACEA_T47D_80hr_Negative	52.79	1.72	--
	NCCT_HEK293T_CellTiterGLO	23.10	1.36	--
Cell Morphology	TOX21_MMP_ratio_down	100.29	2.00	--
	BSK_3C_Vis_down	32.66	1.51	--
Cytokine	BSK_SAg_MCP1_down	27.90	1.45	--
	BSK_SAg_IL8_down	15.67	1.20	--
	BSK_SAg_CD69_down	19.36	1.29	--
	BSK_SAg_CD40_down	27.55	1.44	--
	BSK_SAg_CD38_down	15.28	1.18	--
	BSK_LPS_MCSF_down	18.37	1.26	--
	BSK_LPS_MCP1_down	15.64	1.19	--
	BSK_LPS_CD40_down	11.62	1.07	+
	BSK_KF3CT_MCP1_down	20.04	1.30	--
	BSK_hDFCGF_PAI1_down	27.19	1.43	--
	BSK_hDFCGF_MCSF_down	9.36	0.97	+
	BSK_hDFCGF_IP10_down	12.45	1.10	+
	BSK_CASM3C_IL6_up	37.80	1.58	--
	BSK_4H_MCP1_down	45.68	1.66	--
	BSK_4H_Eotaxin3_down	35.36	1.55	--
	BSK_3C_uPAR_down	33.38	1.52	--
	BSK_3C_MCP1_down	16.53	1.22	--
	BSK_3C_IL8_down	36.94	1.57	--
DNA Binding	TOX21_AhR_LUC_Agonist	41.05	1.61	--
	ATG_MRE_CIS_up	102.82	2.01	--
	ATG_ISRE_CIS_dn	39.23	1.59	--
	ATG_Ahr_CIS_up	2.35	0.37	+
	ATG_E2F_CIS_dn	37.45	1.57	--
	ATG_Xbp1_CIS_up	87.16	1.94	--
	ATG_GATA_CIS_dn	163.60	2.21	--
	ATG_NFI_CIS_up	74.04	1.87	--

IntendedTarget Family	Assay Component Endpoint Name[a]	AC_{50}	Log AC_{50}	True Active[b,c]
	ATG_NRF2_ARE_CIS_up	24.78	1.39	--
	ATG_Sp1_CIS_up	71.50	1.85	--
	ATG_NRF1_CIS_up	163.65	2.21	--
	ATG_Oct_MLP_CIS_up	110.12	2.04	--
	ATG_SREBP_CIS_up	72.41	1.86	--
	ATG_AP_1_CIS_up	44.03	1.64	--
	ATG_BRE_CIS_up	68.25	1.83	--
	ATG_Ets_CIS_dn	102.88	2.01	--
	ATG_GLI_CIS_up	77.08	1.89	--
Esterase	NVS_ENZ_hES	28.56	1.46	--
GPCR	BSK_CASM3C_Thrombomodulin_up	12.27	1.09	+
Ion Channel	NVS_LGIC_rGABAR_NonSelective	12.35	1.09	+
Miscellaneous Protein	BSK_CASM3C_LDLR_up	33.14	1.52	--
Nuclear Receptor	TOX21_TR_LUC_GH3_Antagonist	79.66	1.90	--
	OT_AR_ARSRC1_0960	85.07	1.93	--
	OT_ER_ERaERa_0480	67.01	1.83	--
	OT_ER_ERaERb_0480	64.04	1.81	--
	OT_ER_ERbERb_0480	56.57	1.75	--
	OT_FXR_FXRSRC1_0480	36.33	1.56	--
	OT_NURR1_NURR1RXRa_0480	39.41	1.60	--
	ATG_IR1_CIS_dn	36.94	1.57	--
	ATG_PPARg_TRANS_up	57.24	1.76	--
	ATG_PXR_TRANS_up	4.34	0.64	+
	ATG_ERE_CIS_up	34.33	1.54	--
	ATG_VDRE_CIS_up	4.64	0.67	+
	ATG_ERa_TRANS_up	20.22	1.31	--
	ATG_PXRE_CIS_up	6.34	0.80	+
	ATG_RXRb_TRANS_up	24.10	1.38	--
	ATG_DR4_LXR_CIS_dn	35.16	1.55	--
Oxidoreductase	NCCT_TPO_AUR_dn	16.55	1.22	--
	NCCT_QuantiLum_inhib_dn	41.28	1.62	--
Phosphatase	NVS_ENZ_hDUSP3	9.14	0.96	+
Protease	BSK_hDFCGF_MMP1_up	5.06	0.70	+
	BSK_BE3C_tPA_down	15.26	1.18	--
Steroid Hormone	CEETOX_H295R_TESTO_dn	55.71	1.75	--
	CEETOX_H295R_PROG_up	39.83	1.60	--
	CEETOX_H295R_CORTISOL_dn	82.82	1.92	--
	CEETOX_H295R_ANDR_dn	54.85	1.74	--
	CEETOX_H295R_11DCORT_dn	84.05	1.92	--

a- All assay abbreviations found in http://actor.epa.gov/dashboard2/
b- True actives are assays that are not within the range of cytoxicity (see Figure 8, below). Yellow highlight indicates true positives.
c- Orange highlighted assays are those which show activity for hormone receptors (thyroid, androgen, estrogen) and hormone inhibition (cortisol, progesterone, androgen, testosterone).

The histogram, shown in Figure 7 illustrates the active (true actives + actives: red) and inactive (blue) CPF and CPF-oxon assays along with their intended target families. It is evident that CPF-oxon has more assays that are active in various target families compared with CPF. This would be expected since CPF-oxon is actually the active toxic metabolite and CPF requires metabolic activation which is not provided in the assays. Included in the CPF-oxon active assays are the human and rat AChE inhibition (cell-free) assays in the target family "esterase". These are not active with CPF.


histogram of all tested endpoints for 2921-88-2
# of Active Assays
110
100
90
80
70
60
50
40
30
20
10
1
cell cycle
background measurement
gpcr
protease inhibitor
hydrolase
ion channel
Intended Target Family



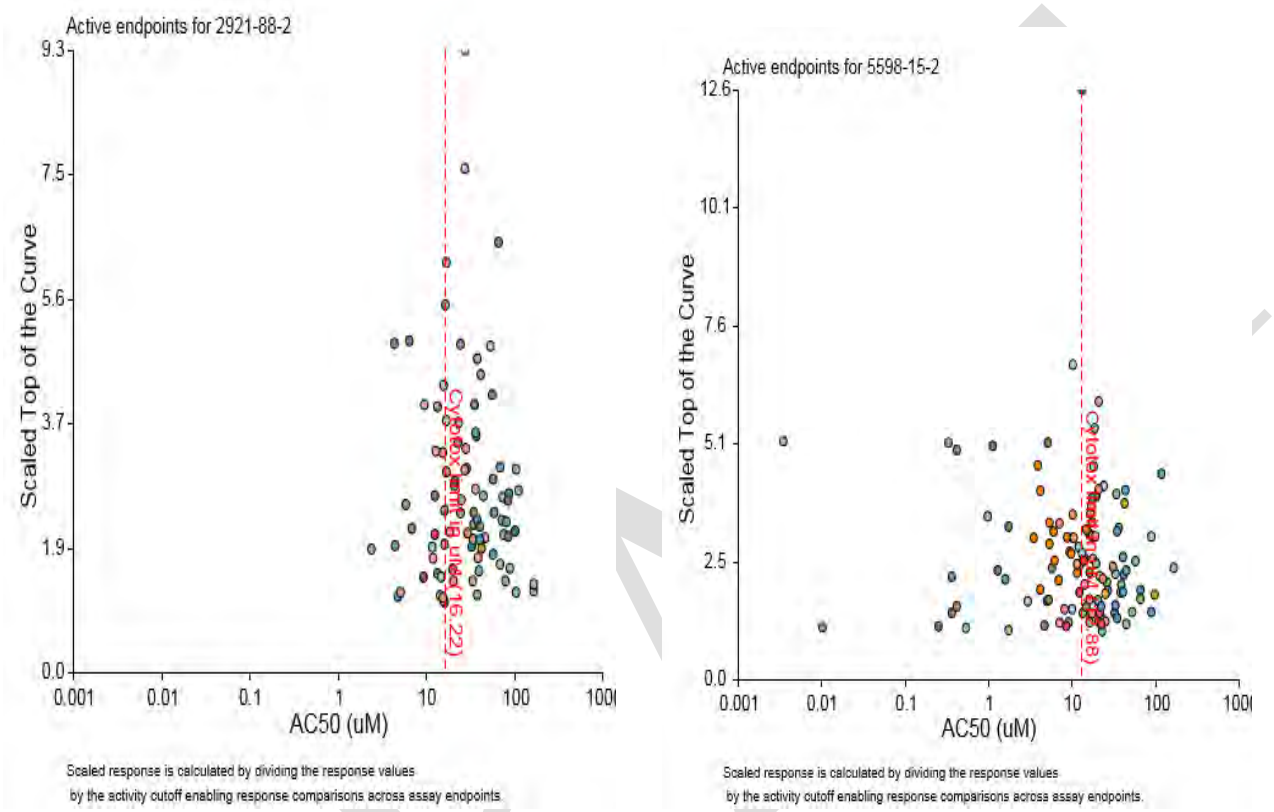
Chlorpyrifos **Chlorpyrifos-oxon**

Figure 7. CPF ToxCast Histograms:
Active (red) and Inactive (blue) ToxCast assays with CPF and CPF-oxon, along with the respective intended target families.

When the assays for each target family are graphed (Figure 8) then the "true actives" for CPF and CPF-oxon can be distinguished from those considered "active" based on a measurable AC_{50} that is above baseline. AC_{50} values appearing to the right of or clustered near the red-dashed line are considered to be within the "burst region" or within the cytotoxicity range. Concentrations for a given compound above or near this range can result in a "burst" of assay activity that can non-specifically stimulate the same cellular reporters used to track the action of specific molecular targets that define the corresponding assay (Browne et al. 2015). More specifically, in receptor-mediated assays the "burst region" represents a grey area where true chemical-receptor interactions and assay interference due to cytotoxicity/apoptosis may result in a false positive response. True active CPF AC_{50} values only occurring beyond the burst region suggest that the metabolic conditions needed to convert CPF to the active, toxic form CPF-oxon (e.g.enzymes or physiological milieu) were not duplicated in the HTS systems used for the *in vitro* assays.

CPF-oxon true actives were in the following component categories: Proteases, background measurements, cell adhesion, cell cycle, cell morphology, cytokine, esterase, gpcr, nuclear receptor, oxidoreductase, protease, protease inhibitor, transferase and transporter (Figure 7and Figure 8). For the CYP assays, there were 13/17 true actives out of the total actives, with 32 total CYP assays performed with CPF-oxon. AChE cell-free, reporter assays with human and rat extracted gene proteins were true actives for

CPF-oxon but not for the parent compound CPF. Both compounds had some activity within the burst region with the estrogen, androgen and thyroid receptor pathways. This indicates the potential for endocrine disruption from CPF exposure at higher doses (all data available at: http://actor.epa.gov/dashboard2/; accessed 12-2015). However, since activity occurs within the burst region for the endocrine assays, the data are equivocal or indicate a secondary pathway.



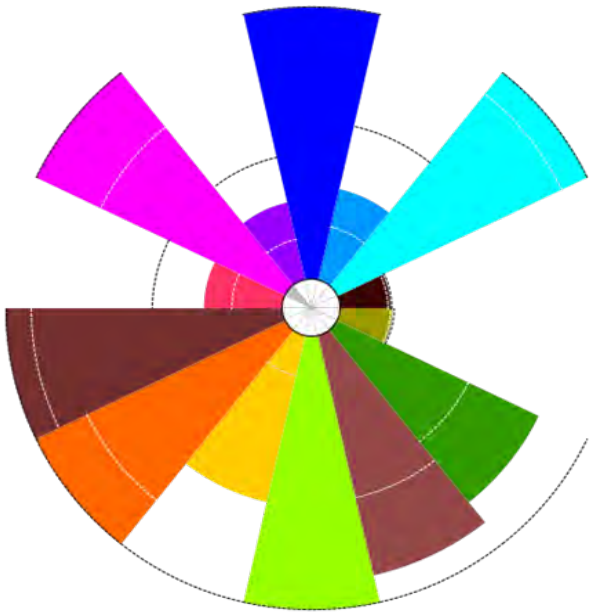
CPF CPF-oxon

Figure 8. True Actives for CPF and CPF-oxon, respectively.
Assays related to each colored dot are on http://actor.epa.gov/dashboard2/

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

The Toxicological Priority Index (ToxPi) "…is a dimensionless index score that is calculated for each chemical as a weighted combination of all data sources that represents a formalized, rational integration of information from different domains. Visually, ToxPi is represented as component slices of a unit circle, with each slice representing one piece (or related pieces) of information" (Reif et al. 2013; UNC 2014). The ToxPi data below in Figure 9 show relative ToxCast assay activities for defined categories between CPF and CPF-oxon. The input data were generated using AC_{50} values for all active assays (i.e., not limited to true actives: ToxCast Dashboard: http://actor.epa.gov/dashboard2/) and "100,000" for inactive assays. The same scaling type ($-\log10^{(x)+6}$) was used for all ToxPi figures shown. The components associated with the various slices are color-coded. The components into which each assay was grouped was from the the ToxCast Dashboard. The Toxicity Scores (Reif et al. 2010; Reif et al. 2013) calculated in the ToxPi program were very similar (9.6 and 12 for CPF and CPF-oxon, respectively), however the ToxPi figures below show the

relative toxicities for each component compared. Based on the Toxicity Score, CPF-oxon would be more of a priority for further examination of toxicity than its parent compound. The components compared below are only for actives as defined on the ToxCast Website but was not broken down into ToxPi for true actives.



CPF

Overall ToxPi score: 9.629

Scores calculated relative to 2 number of substances

- transporter
  - Value: 0.183 CI=[0.173:0.189], Scaling: -log10(x)+6, Missing data: 0.0%
- cell adhesion
  - Value: 1.0 CI=[0.897:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- gpcr
  - Value: 0.346 CI=[0.203:0.579], Scaling: -log10(x)+6, Missing data: 0.0%
- steroid hormone
  - Value: 1.0 CI=[1.0:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- esterase
  - Value: 0.296 CI=[0.16:0.465], Scaling: -log10(x)+6, Missing data: 0.0%
- cytokine
  - Value: 1.0 CI=[0.743:1.0], Scaling: -log10(x)+6, Missing data: 4.348%
- background measurement
  - Value: 0.287 CI=[0.189:0.473], Scaling: -log10(x)+6, Missing data: 0.0%
- dna binding
  - Value: 1.0 CI=[0.908:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- protease
  - Value: 1.0 CI=[0.797:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- cell morphology
  - Value: 0.625 CI=[0.151:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- oxidoreductase
  - Value: 1.0 CI=[1.0:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- nuclear receptor
  - Value: 0.898 CI=[0.609:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- cell cycle
  - Value: 0.805 CI=[0.524:1.0], Scaling: -log10(x)+6, Missing data: 0.0%
- cyp
  - Value: 0.191 CI=[0.184:0.196], Scaling: -log10(x)+6, Missing data: 0.0%

Missing data in percent:

0 0 0.01 0.1 1 10 20 30 40 50 60 70 80 90 100

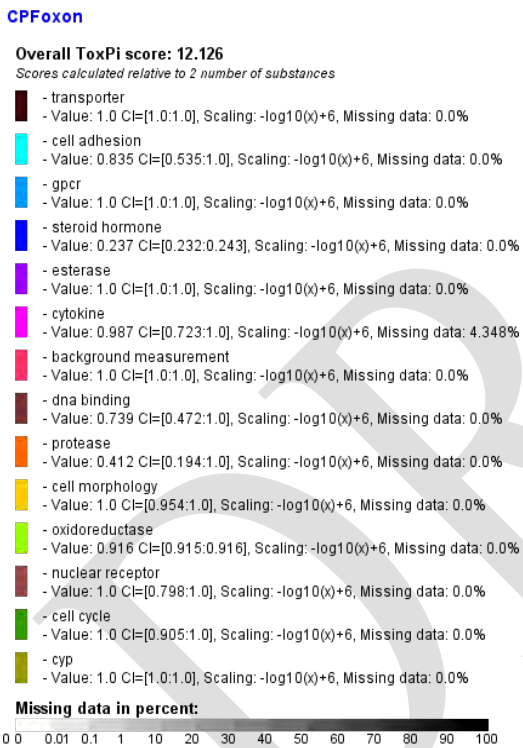


Figure 9. Toxicology Priority (ToxPi):
The ToxPi scale measured the presumptive components showing ToxCast assay activity with CPF (A) and CPF-oxon (B).

II.L.4. U.S.EPA ToxCast Assays in Zebrafish

Zebrafish (ZF: *Danio rerio*) provide a model for studying effects of CPF *in vivo*. They share many developmental, anatomical, and physiological characteristics with mammals since molecular signaling is conserved across species (Padilla et al. 2012; Padilla et al. 2011; Sipes et al. 2011; Tanguay 2013; Tanguay

et al. 2013). They also require AChE for normal neurodevelopment (Behra et al. 2002a). For that reason, ZF are useful for studies of neurobehavioral developmental effects of AChE inhibitors like CPF.

ZF embryos can reveal acute toxic effects of CPF since growth and development occur at such a rapid rate. Therefore, if a chemical is developmentally toxic in ZF, it would affect molecular pathways or processes that might be detected by phenotypic and/or neurobehavioral responses. These changes can then serve as indicators of affected pathways for target identification (Padilla et al. 2012; Padilla et al. 2011; Tanguay et al. 2013; Truong et al. 2014). The two primary models in ZF consist of using either intact embryos (Padilla et al. 2012) or using embryos with the chorion removed (Tanguay et al. 2013) (http://actor.epa.gov/dashboard2/).

II.L.4.a. Zebrafish Method of 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 hpf (hours post-fertilization: 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: 20=non-hatching; 40=lethality) 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 = 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.

II.L.4.b. Zebrafish Method with Chorion Removed (Tanguay et al. 2013; Truong et al. 2014)

Tanguay et al. (2013) removed the chorion from the ZF 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. ZF (32/concentration) were treated with the test chemical at 0.064–640µM (10-fold serial dilutions) in DMSO (0.64% v/v). A positive control (5 ul trimethyltin chloride) was also used. ZF were dosed 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" (i.e. only one dose of test compound) exposed 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).

Padilla et al. (2012) indicated that the AC_{50} (concentration at 50% activity) for CPF (8.5 µM) was 21-fold greater than the AC_{50} for CPF-oxon (0.40 µM). Their Terata Scores (sum of all malformations and variations), were identical (40: highest score possible) which means that the chemicals are ultimately embryotoxic. It also suggests that the ZF liver was able to metabolize CPF to the oxon form. The slope was very steep for CPF between AC_{10} (3.0 µM) which is considered to be a NOEL and the AC_{50} (8.5 µM).

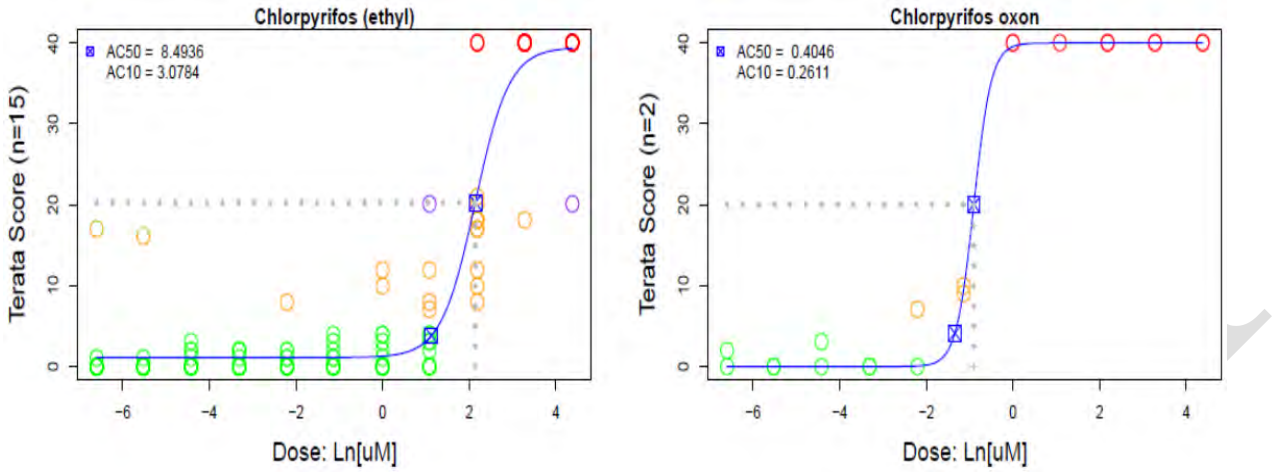


Figure 10. Terata Scores for CPF
Green = control levels; red = dead (Terata Score=40); purple= not hatched but alive (Terata Score ~ 20); yellow = animals alive and hatched (Terata score 8-20).

II.L.4.c. Zebrafish Results (Tanguay)

The graphs shown below indicated the individual malformations by chemical (Figure 1). Unlike what was observed with the Padilla method, 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 uM; pericardial edema (PE) and caudal fin (CF) abnormalities occurred at 64 uM. With the Tanguay method, ZF apparently do not have the metabolic capability to produce a sufficient quantity of the oxon to cause the overt oxon-mediated toxicity (Yang et al. 2011). It's also possible that CPF is not actually getting into the animals to be metabolized.

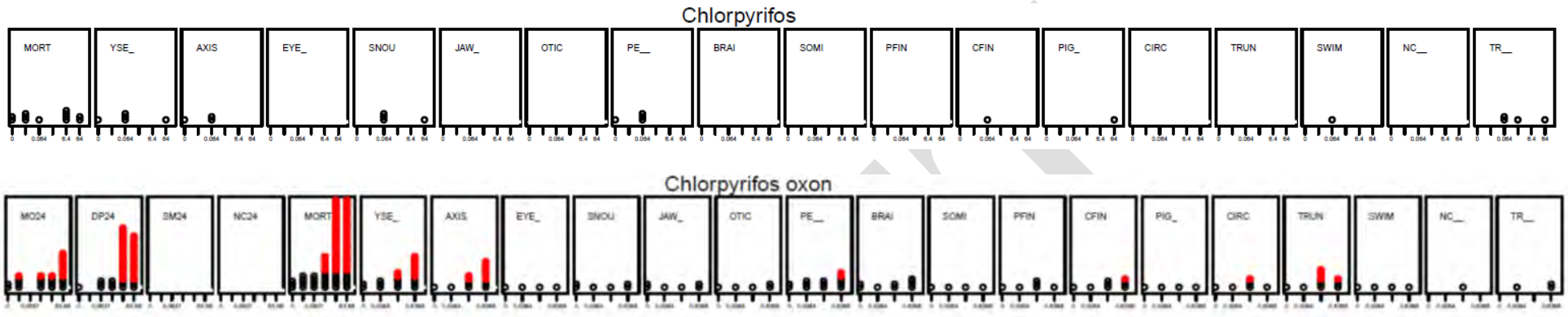


Figure 11. Morphological effects from CPF or CPF-oxon treatment in zebrafish.
There were no effects for CPF.CPF-oxon caused mortality at 24 hours at all doses (MO24); developmental progress (inhibited) at 24 hours (DP24); mortality (mort); yolk sac (YSL), axis and trunk abnormalities were observed at ≥ 6.4 µM; pericardial edema (PE) and caudal fin (CF) abnormalities occurred at 64 µM.

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

Levin et al. (2003) used CPF at 0.01 and 0.10 µg/ml (DMSO vehicle) on ZF embryos (chorion intact) for 5 days. Animals were tested for behavioral effects intermittently up to 26 weeks. Mortality was high at 0.10 µg/ml (5/12 died) at 38 weeks (0/13 DMSO; 1/16 0.01 µg/ml). At 0.01µg/ml, ZF 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 ZF for effects of CPF on swimming behavior. Tested at day 6, animals showed decreased swimming activity and decreased habituation of swimming activity at 0.10ug/ml. These effects involve the central nervous system (CNS: ≥0.01 µg/ml) as well as peripheral nervous system (PNS: 0.10 µg/ml: muscular).

ZF embryos (chorion intact) were treated with 0.10 ug/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," decreased escape diving response, increased swimming activity and lower learning rate. When **placed in a new environment** (novel tank exploration test) the ZF also showed a decrease in escape diving response and increased swimming after 5 days of treatment.

Jin et al. (2015) showed neurobehavioral (swimming activities and behaviors related to stimulation of light/dark photoperiod transition) and teratogenic effects (spinal deformities, spinal deformities, pericardial edema) in ZF (chorion intact) after CPF treatment to dechorionated embryos at

0.10 µg/ml for 5 days. Results indicated that neurobehavioral effects occurring after treatment with CPF in an embryonic stage were not reversible. In addition, AChE inhibition, oxidative stress-related enzyme levels and the transcriptional levels of genes related to neurotoxicity were affected.

CPF was shown to affect anxiety-related behaviors in ZF (chorion intact) at ≥0.01µM when they were exposed for 7 dpf (Richendrfer et al. 2012a). The altered behaviors exhibited included decreased swim speed and thigmotaxis (edge preference) without changes in avoidance behavior. At 0.001 µM CPF, there were no changes in swim speed, thigmotaxis, or avoidance behavior and at 1 µM CPF there were both behavioral and teratology effects. Thigmotaxis is an anxiety-related behavior in ZF larvae (Richendrfer et al. 2012b) and this behavior alteration appears to be directly related to exposure to low doses of CPF.

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

AChE activity is critical to ZF nervous system development as has been demonstrated by Behra et al. (2002a). They developed a genetically altered ZF strain (*ache: chorion intact*) which eliminated AChE activity and the phenotype displayed disruptions in both neural (PNS) and muscle fiber development (Behra et al. 2002b). Initially the embryos are motile but then primary sensory neurons die, resulting in defective innervation of muscle fibers which 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. 2002b). The authors concluded that loss of AChE activity in the mutant ZF resulted in hyperstimulation of the muscle fibers, which led to fiber disruption and degeneration.

Yen et al. (2011) examined the possibility that the CPF MOA also involves inhibition of ZF AChE resulting in hyperstimulation at cholinergic synapses and subsequent loss of neuromuscular activity by neuronal death. They examined AChE inhibition in ZF embryos (intact chorion) after exposure to 0.30 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 ZF movements were tracked at 6 dpf (one day after 0-5 dpf exposure). At 0.30 µM CPF exposures reduced locomotor activity by 35% 0.30 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 ZF (chorion intact) treated at lower doses of CPF (0.001, 0.01, 0.1 µM or ~0.00035, 0.0035, 0.035 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 0.30 µM (0.105µg/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 CPF, whereas at ≥0.01 µM CPF there was a significant increase in abnormal behavioral ("fish at rest" was increased; swim speed was decreased after 1-5 dpf treatment). ZF 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) (Richendrfer et al. 2012a, 2012b) at ≥0.01 µM 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 ZF during development.

III. HAZARD IDENTIFICATION

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

III.A. Acute Toxicity

The profile of acute CPF toxicity has been extensively described (Eaton et al. 2008; Koshlukova and Reed 2014; Testai et al. 2010). The database for the acute toxicity for CPF consists of Health Effects Test Guideline studies submitted to CDPR by registrants (see APPENDIX 1 for HHAB one-liners) 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 (Eisler 2007). The oral, dermal and inhalation LD_{50}s; dermal and eye irritation, dermal sensitization and acute delayed neurotoxicity studies using technical CPF were previously shown (Table 6).

III.A.1. Acute 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 inihibition.

Carr et al. (2013); Carr et al. (2014) were the only studies reporting overt toxicity with the same NOEL as that occurring for AChE inhibition (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 [monoacylglycerol lipase (MAGL), and fatty acid amide hydrolase (FAAH)] involved in endocannabinoid degradation. The associated neuromodulatory lipid endocannabinoids were 2-arachidonoylglycerol (2-AG) metabolized by MAGL and anandamide (AEA) 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 level (Anavi-Goffer and Mulder 2009; Harkany et al. 2008). 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 preliminary studies by Carr et al. (2015) and Mohammed et al. (2015) which showed significant neurobehavior 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 available about this system to use it for a critical NOEL. Instead these effects will be evaluated in relation to database uncertainties for potential increased sensitivity in infants and children.

It is notable that the majority of the acute CPF oral studies performed in rat, mouse, rabbit and human have similar NOELs (Table 13, Table 14). The lowest acute CPF NOELs are based on AChE inhibition (RBC or plasma ChE) for rats, mice, rabbits and humans. Low acute CPF NOEL values ranged from 0.05 mg/kg/d in rat pups (Marty and Andrus 2010) based on decreased plasma ChE on PND 11 to 0.75 mg/kg/d in rat adults receiving a single dose administration PND 7 with decreased plasma ChE inhibition (Zheng et al. 2000). Brain ChE NOELs ranged from 0.3 mg/kg/d in pregnant rats (Hoberman 1998) to 0.5 mg/kg/d in pre-weanling and young adult rat pups (Marty and Andrus 2010; Marty et al. 2007). Therefore in acute oral animal studies, the lowest NOELs, based on BuChE (plasma 0.05 mg/kg/d), identify the most sensitive endpoint for CPF.

The acute oral NOELs (or PoDs) used by the U.S.EPA were obtained from their PBPK-PD model based on 10% RBC AChE inhibition data from human studies (Kisicki et al. 1999; Nolan et al. 1984; Smith et al. 2014; Smith et al. 2011). 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. In addition, the current 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; Hinderliter et al. 2011; Lowe et al. 2009; Poet 2013; Poet et al. 2014; Smith et al. 2014; Smith et al. 2011; Timchalk et al. 2002a; Timchalk et al. 2002b; Timchalk et al. 2006), reviewed by stakeholders, the SAP and the U.S.EPA (U.S. EPA 2014a). Use of human data and thorough vetting of the model potentially decreases the interspecies uncertainty to 1x. Therefore, HHAB used the PoDs in Table 20 for acute oral CPF exposures in infants <1 yr old (0.60 mg/kg/d), young children 1-2 yrs old (0.581 mg/kg/d), children 6-12 yrs old (0.53 mg/kg/d), youths 13-19 yrs old (0.475mg/kg/d) and females 13-49 yrs old (0.457 mg/kg/d). The lowest acute oral PoD (0.457 mg/kg/d) for females 13-49 years old (women of childbearing age) will be used for dietary exposure assessments.

For acute oral spray-drift risk characterization, the ***steady-state PoD*** for children (1-2 yrs old: 0.099 mg/kg/d) was used (Table 20). 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 the inhibition is still 50% in plasma or ~20% in RBC (AChE) resulting in accumulated inhibition in those exposed for 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). (Hoberman 1998; Marty and Andrus 2010; Mattsson et al. 1998; Maurissen et al. 2000); Nolan et al. (1982); (U.S. EPA 2011a) showed no AChE inhibition in human plasma ChE after a single treatment at a single dose (5.0 mg/kg/d). No overt effects were reported in either study. 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 U.S.EPA Health Effects Test Guidelines and the toxicological significance of plasma and RBC AChE inhibition by itself is uncertain, especially in animals compared to humans. Therefore, HHAB used the PBPK-PD-generated steady-state dermal PoDs of 11.89 mg/kg/d for females (13-49 years old) and 134 mg/kg/d for children (1-2 yrs old) to evaluate the acute spray-drift dermal exposure scenarios (Table 20).

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 ($BMDL_{10}$ = mg/m^3; 0.09 ppm or 0.89 mg/kg/d: Hotchkiss et al., 2010). A BMD analysis was performed (U.S.EPA BMD Software version 2.6; Hill model) for RBC AChE inhibition in rat at 48 hrs post-dose because a NOEL was not achieved and Hotchkiss et al. (2010) was the only inhalation study performed with aerosol. 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 aerosol, since that is the most likely media form for human inhalation exposure in California (Kwok, 2015; APPENDIX 3). Because CPF in aerosol form is the most likely route of exposure in California, the 0.09 ppm will be used for evaluating acute spray-drift inhalation exposure. The BMDL for this was 3.17 mg/m^3 (0.89 mg/kg/d) based on ChE inhibition in male and female rats (Hotchkiss et al. 2010). The BMD modeling was performed by U.S.EPA Benchmark Dose Software version 2.6 (Hill model, 95th percent confidence limit).

The PBPK-PD model for inhalation exposure incorporated data from the rat aerosol inhalation study Table 8 (Hotchkiss et al. 2010). According to the U.S.EPA 2014 IRED "The PBPK-PD model predictions for rats inhaled CPF compared well with animal data (Hotchkiss et al. 2013) with respect to CPF, oxon, and TCPy concentrations in plasma, and ChE in plasma, RBC and brain (Poet et al. 2014)." The U.S.EPA did not anticipate acute inhalation exposure for their residential scenarios. They instead generated PoDs for steady-state inhalation exposure for two critical subpopulations (children 1-2 years-old: 0.00237 mg/m^3; females 13-49 years-old: 0.00615 mg/m^3) (U.S. EPA 2014a).

Acute inhalation exposure scenarios from CPF spray-drift occur in California and the subgroups anticipated to be most sensitive are females (13-49 yrs old: 0.00615 mg/kg/d) and children (1-2 years old: 0.00237 mg/m^3).

III.B. Subchronic Toxicity

Subchronic CPF toxicity was described and reported in the U.S.EPA RED and IREDs (U.S. EPA 2007, 2011a, 2014a) and in the HHAB Summary of Toxicology Data (APPENDIX 1). Registrant-submitted studies under consideration for the subchronic endpoints are in Table 18, below (Boverhof et al. 2010; Breslin et al. 1991; Marable et al. 2001; Maurissen et al. 1996; Shankar et al. 1993; Szabo et al. 1988). All are considered acceptable according to U.S.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

Subchronic studies available for CPF endpoint determination are shown below in Table 18. Overt subchronic effects from CPF treatment included reduced body weights and feed consumption, increased clinical signs and 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, 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 U.S.

EPA (2011a) from a study performed in pregnant rats treated GD 6-20 (Hoberman 1998; Marty and Andrus 2010; Mattsson et al. 1998; Maurissen et al. 2000; U.S. EPA 2011a).

The U.S.EPA calculated an oral steady-state (21-day) PoD of 0.078 mg/kg/d from the PBPK-PD model. This value is preferable to the BMDL obtained from animal models because the data were obtained from human studies (Kisicki et al. 1999; Nolan et al. 1987; Nolan et al. 1984; Rauh et al. 2011; Smith et al. 2014; Smith et al. 2011) and because the PBPK-PD model has been thoroughly evaluated by several sources, including publication of the model in peer-reviewed journals, reviewed by stakeholders, the SAP and the U.S.EPA (U.S. EPA 2014a). Because of this, the uncertainty about interspecies variability is reduced. HHAB will use the steady-state PoDs in Table 20 for oral CPF exposures in infants <1 yr old (0.103 mg/kg/d), young children 1-2 yrs old (0.099 mg/kg/d), children 6-12 yrs old (0.090 mg/kg/d), youths 13-19 yrs old (0.080 mg/kg/d) and females 13-49 yrs old (0.078 mg/kg/d). The lowest steady-state oral PoD (0.078 mg/kg/d) for females 13-49 years old (women of childbearing age) will be used for subchronic/chronic dietary.

As discussed above (III.A.1. Acute Oral Toxicity) the oral steady-state PoDs 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 (only dose tested) (Calhoun and Johnson 1988) (Table 18). Nor was a NOEL achieved in another CPF dermal study performed in mice (Krishnan et al. 2012) where the LOEL was 101 mg/kg/day 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 the U.S.EPA predicted steady-state 10% RBC AChE inhibition based on TCPy as a biomarker for CPF exposure in humans (Lowe et al. 2009; Poet et al. 2003; Smith et al. 2014; Smith et al. 2011; Timchalk et al. 2007; Timchalk and Poet 2008). The modeled steady-state dermal PoDs are therefore useful to HHAB 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 (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 [highest dermal exposure]) (U.S. EPA 2014a) Table 20. As discussed above (III.A.2. Acute Dermal Toxicity) the dermal steady-state PoDs for females (13-49 yrs old: 11.89 mg/kg/d) and children (1-2 yrs old: 134 mg/kg/d) were also used to assess acute spray-drift risk.

III.B.3. Subchronic Inhalation Toxicity

A 13-week study by Newton (1988) in rats achieved a NOEL (0.010 ppm; 0.143 mg/m^3) based on decreased AChE activity (Table 18). Although this was an acceptable subchronic inhalation study, it was performed with CPF vapor and not with an aerosol. The U.S. EPA (2014a) reported PoDs for steady-state (subchronic 21-day) inhalation exposure for two critical subpopulations (children 1-2 years-old: 0.00237 mg/m^3; females 13-49 years-old: 0.00615 mg/m^3)(U.S. EPA 2014a). These PoDs were selected to as the critical NOELs to be used to evaluate subchronic spray drift inhalation exposure to CPF (Table 20). As discussed above (III.A.3. Acute Inhalation Toxicity) the inhalation steady-state PoDs for females (13-49 yrs old: 0.00615 mg/m^3 mg/kg/d) and children (1-2 yrs old: 0.00237 mg/m^3) were also used to assess acute spray-drift risk.

Table 18. 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** **ChE 0.05**	Overt 5.0 AChE 0.1	1*
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.U.S. EPA (2011a), Mattsson et al. (1998); Maurissen et al. (2000), Marty and Andrus (2010) 8. Calhoun and Johnson (1988); 9. Krishnan et al. (2012); 10. Landry et al. (1986); 11. Corley et al. (1986); 12. Newton (1988)
Abbreviations: AChE: cholinesterase; RBC: red blood cell

III.C. Chronic Toxicity

Chronic CPF toxicity was described and reported in the U.S.EPA RED and IREDs (U.S. EPA 2007, 2011a, 2014a) and in the HHAB Summary of Toxicology Data (APPENDIX 1). Registrant-submitted studies under consideration for the chronic endpoints are in Table 19 below (Crown 1990; Gur 1992; McCollister et al. 1971; Young and Grandjean 1988a). All are considered acceptable according to U.S.EPA Health Effects Test Guidelines (U.S. EPA 2000a).

III.C.1. Chronic Oral Toxicity

Chronic studies available for CPF endpoint determination show that the most sensitive endpoint in rats (Crown 1990; Young and Grandjean 1988a), mice (Gur 1992) and Beagle dog (McCollister et al. 1971) was ChE inhibition (Table 11 and Table 12). An BMD_{10}/$BMDL_{10}$ for RBC AChE inhibition was estimated for pregnant female rat ($BMDL_{10}$ =: 0.03 mg/kg/d) by the U.S.EPA in their 2011 IRED (U.S. EPA 2011a) based on data from Hoberman (1998), Mattsson et al. (1998), Maurissen et al. (2000) and Marty and Andrus (2010).

Overt chronic effects from CPF treatment (Table 19) included reduced body weight, reduced food and water consumption, yellow perineal stain, increased clinical signs; 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 (Crown 1990; Young and Grandjean 1988a). The NOELs for these overt effects were at doses higher than those for AChE inhibition.

The steady-state PBPK-PD model used by the U.S. EPA (2014a) (described previously) was based primarily on 10% RBC AChE inhibition in humans. The PBPK-PD steady-state PoDs described earlier (III.B.1 Subchronic Oral Toxicity) also applied to chronic exposure (Table 20). Although steady-state values are higher than the $BMDL_{10}$ estimated 0.03 mg/kg/d, they are based on human data in a well-vetted model. HHAB used the steady-state PoDs for oral CPF exposures in infants <1 yr old (0.103 mg/kg/d), young children 1-2 yrs old (0.099 mg/kg/d), children 6-12 yrs old (0.090 mg/kg/d), youths 13-19 yrs old (0.080 mg/kg/d) and females 13-49 yrs old (0.078 mg/kg/d). The lowest steady-state oral PoD (0.078 mg/kg/d) for females 13-49 years old (women of childbearing age) 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 (rationale described earlier).

III.C.2. Chronic Dermal Toxicity

There were no chronic dermal toxicity studies available for CPF (Table 19). The U.S.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]) (U.S. EPA 2014a). Since CPF RBC AChE inhibition reaches a steady-state within a 21 d period, HHAB selected the children 1-2 yrs-old: 134.25 mg/kg/d and females 13-49 yrs-old: 23.6 mg/kg/d PoDs 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 19). The U.S. 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: 0.00237 mg/m^3; females 13-49 years-old: 0.00615 mg/m^3)(U.S. EPA 2014a). Once again, since the steady-state for ChE inhibition is achieved within 21 days. Therefore, the steady-state modeled PoDs were selected by HHAB to evaluate chronic inhlation exposure from CPF spray drift (Table 20).

Table 19. Chronic AChE and Overt Effects of CPF and the Respective NOELs and LOELs

Species	Exposure Duration	Effects	NOEL	LOEL	Ref[a]
			mg/kg/day		
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 (1988a); 2. Crown (1990); 3. McCollister et al. (1971); U.S. EPA (2011b); 4. Gur (1992); 7. U.S. EPA (2011a), Hoberman (1998); Mattsson et al. (1998); Maurissen et al. (2000), Marty and Andrus (2010)

*The study was acceptable to HHAB based on FIFRA guidelines

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

Table 20 shows a summary of the critical NOELs and endpoints selected for evaluating oral, dermal and inhalation exposure from diet and spray drift. The PBPK-PD model is currently 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; 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 incorporated acute oral and 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) (U.S. EPA 2014a). Based on the above, the PBPK-PD modeled values from U.S.EPA 2014 risk assessment were used for HHAB'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 20. Summary of Critical NOELs for All Exposure Durations Used for HHAB's Risk Assessment

Exposure Route[a]	PBPK-PD PoDs (U.S.EPA, 2014)									
	Infants < 1 yr old		Children 1-2 yrs		Child 6-12 yrs old		Youths 13-19 yrs old		Females 13-49 yrs old	
	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]	Acute	SS[b]
Drinking Water or Dietary (food only) Exposure										
Drinking H_2O (oxon ppb)	1,183	217	3,004	548	7,700	1,358	4,988	878	5,285	932
Food (mg/kg/d)	0.600	0.103	0.581	0.099	0.530	0.090	0.475	0.080	0.467	0.078
Spray Drift Exposure to Bystanders										
Oral (mg/kg)	--	--	--	0.099	--	--	--	--	--	--
Dermal (mg/kg/d)	--	--	--	134.25	--	--	--	--	--	23.60
Inhalation (mg/m^3)	--	--	--	2.37	--	--	--	--	--	6.15

a- Parent compound CPF for all estimates but drinking water where CPF-oxon exposure is estimated.
b- SS = Steady-state: HHAB used SS oral, dermal and inhalation PoDs, since crop treatment occurred at 10 day intervals and plasma ChE and RBC AChE inhibition takes approximately 26 -days to reverse to normal values (Nolan et al. 1984).

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 involve both aerial and ground-based methods, and the latter includes groundboom and airblast (Dawson et al. 2012). For agricultural applications, there are 21 products currently registered in California with formulations including aqueous concentrate, emulsifiable concentrate, wettable power, and liquid (Table 21). In this exposure assessment, granular products are omitted because the focus is on spray drift following application of a liquid.

Table 21. CPF Products labeled for Use in the Production of An Agricultural Commodity in California

Product Name	EPA Registration No.	Formulation
Agrisolutions Yuma 4E Insecticide	62719- 220-AA- 1381	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
Dursban 50w In Water Soluble Packets	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 Insecticide	279- 9545-AA	Emulsifiable Concentrate
Vulcan	66222- 233-AA	Other(Liquid)
Warhawk	34704- 857-AA	Aqueous Concentrate
Warhawk	34704- 1077-AA	Emulsifiable Concentrate
Whirlwind	62719- 220-AA- 5905	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 (Table 22), short-term exposure is determined to be the focus of this bystander exposure assessment due to spray drift. CDPR 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. 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 U.S. 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 groundboom and orchard airblast applications and AGDISP (AGricultural DISPersal near-wake Lagrangian model version 8.28) for aerial applications (Barry 2015). For the post-application assessment, U.S. EPA standard operating procedures (SOP) for residential exposure assessment were followed (U.S. EPA 2013b).

Technical description of these models has been detailed elsewhere (Barry 2015; Teske et al. 2002a; Teske et al. 2002b). Both AgDRIFT and AGDISP models predict the off-site deposition of CPF products occurring relative to the nominal application rate (i.e., drift fraction) at different distances downwind: 1000 feet for the aerial and 300 feet for groundboom and airblast applications. Table 22 shows the application types and model parameter values for use in estimating the drift deposition. These scenarios and parameter values were chosen to maximize the drift deposition estimates from spray drift under different application types. To ensure the deposition estimates are consistent with the application methods of airblast and groundboom in California, the number of swaths employed was 40 for the former and 60 for the latter instead of the default (i.e., 20 swaths) in AgDRIFT. In addition to the deposition estimates, for the aerial applications, one hour time-weighted average air concentrations (unit mg/m^3) of CPF at vertical heights of 1.7 ft and 5 ft (i.e., breathing zone heights) were generated by AGDISP for use in estimating inhalation exposure of small children and adults, respectively. Similar to the deposition estimates, these time-weight air concentrations are the highest possible air concentrations based on the parameters listed in Table 22.

Table 22. Application Type Scenarios for Chlorpyrifos Deposition Estimates (Barry 2015)

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)
Groundboom	Low Boom	20 inches above the canopy	M-to-C	40 (37.2)
	High Boom	50 inches above the canopy	M-to-C	40 (37.2)
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.

Table 23 shows the single application rate (unit: pound per active ingredient per acre [lb a.i./acre]) grouping of CPF products registered in California; this table is modeled after the U.S. EPA spray drift exposure assessment document (Dawson et al. 2012). These application rates were used for translating the drift fraction outputs of AgDRIFT and ADISP models into exposure estimates.

Table 23. Application Rates Grouping of Chlorpyrifos Usages in California[a]

Single Application (lb a.i./acre)	Example Use Site	Example Product	Comments
	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.

Evaluation of dermal and inhalation exposures of non-occupational bystanders to spray drift was based on a modified U.S. EPA residential SOP which incorporated off-site movement of pesticide from the results of AgDRIFT and AGDISP models (U.S. EPA 2012; U.S. EPA 2013). Briefly, non-occupational 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 drift deposit and (or) directly via inhalation of the airborne material (e.g., aerosol).

For assessing indirect exposure to spray drift for adults and small children, the residential turf post-application SOP is considered by the U.S. EPA as the standard method (U.S. EPA 2013). That is, activities of adults and children on the contaminated lawn may result in transfer of drift deposit from different surfaces to their skin. In addition to the contact exposure via skin, exposure to the drift deposit may occur via mouthing such as hand-to-mouth, object-to-mouth, and incidental soil ingestion for small children. In this exposure assessment, females of 13-49 years are a primary focus because of their potential increase in susceptibility to the toxicological effects of CPF during pregnancy. The U.S. EPA residential SOP identifies activity patterns associated with children in the 1-2 year old life-stage as resulting in the highest exposure potential to CPF residue on: 1) turf; 2) contaminated lawn via direct dermal contact and (or) mouthing such as hand-to-mouth, object-to-mouth, and 3) 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 ($\mu g/cm^2$)
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 U.S. EPA 2012 residential SOP (U.S. EPA 2012), for assessing individual exposure of pesticide on turf, chemical-specific TTR on the day of application ($TTR_{Day\ 0}$) should be used 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:

$$TTR_{Day\ 0} = \left(\frac{TTR_{expt} \times AppRate_{target}}{AppRate_{expt}}\right) \times F$$

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 a.i./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 a.i./acre) produced by AgDRIFT or AGDISP models as transferable residue following application

For estimating exposures to drift deposit due to mouthing activities of small children (i.e., hand-to-mouth, object-to-mouth, and incidental soil ingestion), computational methods as defined in the U.S. EPA residential SOP were strictly followed (U.S. 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 generation of the needed breathing zone air concentrations. Accordingly, inhalation exposure calculations were performed only for the aerial application of CPF.

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

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

Table 24 and Table 25 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 females of 13-49 years old and children of 1-2 years old, respectively, due to aerial applications at three application rates with two types of aircraft: fixed-wing (AT802A airplane) and rotor-wing (Bell 205 helicopter). As can be seen in Table 24 and Table 25, increases in CPF application rate resulted in a corresponding increase in the 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 drift deposition and inhalation exposure estimates, an additional AGDISP simulation was performed. As shown in Table 25, for a given application rate, the drift exposure estimates appear to be insensitive to the change in spray volumes. By contrast, 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 APPENDIX 3 (Barry 2015).

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

Table 26 shows the drift exposure estimates (in μg/kg/day) of CPF for females of 13-49 years old at four allowable application rates with two ground-based application methods: groundboom and airblast. For groundboom, drift deposition estimates were derived using two swath percentiles: 50^{th} and 90^{th} percentiles (SEE APPENDIX B within APPENDIX 3 for rationale of calculations (Barry 2015)). Table 28, Table 29 and Table 30 show the drift exposure estimates of CPF for children aged 1-2 years old: groundboom (Table 28, Table 29) and airblast (Table 30). For both of these application methods and population subgroups, as expected, the drift exposure estimates increase with the application rates of CPF. The higher drift exposure estimates of the high-boom compared to the low-boom are consistent with the difference in canopy interception between the two elevations. Also, the higher drift exposure estimates with orchard airblast compared to groundboom are consistent with the lower spray interception from low canopy density found in dormant apple and sparse orchards compared to normal orchards.

Table 24. Estimated Doses via Dermal and Inhalation for Females (13-49 Years Old) at Various Distances from a Treated Field 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,b]	1	24.1912	20.6292	16.2365	10.9376	5.7017	3.3983	1.8125
		2	48.5586	41.2836	32.3724	21.6235	10.8244	5.8905	2.3159
		2.3	55.8424	47.4472	37.1704	24.7802	12.3901	6.5714	2.5475
Bell 205 Helicopter	2	1	30.8872	19.5468	11.9697	7.2750	4.6444	2.7564	1.3468
		2	62.2023	39.6222	24.3926	15.1793	8.4078	4.3046	1.8880
		2.3	71.5616	45.5945	28.0804	17.5141	9.4952	4.7766	2.0554
1-Hour Air Concentration at Various Distance Downwind from the Treated Fields (mg/m^3)									
			10 (feet)	25 (feet)[c]	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	0.032	0.0259	0.0174	0.0111	0.0052
		2.3	0.0428	0.0393	0.0341	0.0275	0.0183	0.0115	0.0054
Bell 205 Helicopter	2	1	0.0288	0.024	0.0197	0.0158	0.0111	0.0074	0.0042
		2	0.05	0.0404	0.0322	0.0246	0.0154	0.0093	0.0049
		2.3	0.0538	0.0435	0.0344	0.026	0.016	0.0096	0.005

a- Minimum spray volume as specified on CPF product label for the aerial application.
b- No risk estimate was performed with spray volume of 15 gallons/acre.
c- Buffer zone of 25 feet is required for aerial application of CPF.

Table 25. Dermal and Oral Doses 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 (mg/kg/d)						
				10 feet	25 feet	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	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	Dermal	1	33.45	28.30	21.99	14.54	7.56	4.91	3.56
			2	68.60	58.30	45.75	30.74	16.05	10.44	7.49
			2.3	79.78	67.85	53.21	35.77	18.93	12.22	8.70
		Object-to-Mouth	1	0.021	0.018	0.014	0.009	0.005	0.003	0.002
			2	0.044	0.037	0.029	0.020	0.010	0.007	0.005
			2.3	0.051	0.043	0.034	0.023	0.012	0.008	0.006
		Hand-to-Mouth	1	0.696	0.589	0.458	0.302	0.157	0.102	0.074
			2	1.427	1.213	0.952	0.639	0.334	0.217	0.156
			2.3	1.660	1.412	1.107	0.744	0.394	0.254	0.181
		Soil Ingestion	1	0.0052	0.0044	0.0034	0.0023	0.0012	0.0008	0.0006
			2	0.0106	0.0091	0.0071	0.0048	0.0025	0.0016	0.0012
			2.3	0.0124	0.0105	0.0083	0.0056	0.0029	0.0019	0.0014
Bell 205 Helicopter	15	Dermal	1	43.15	26.66	15.61	9.08	6.16	4.65	3.10
			2	87.41	54.39	32.29	19.04	13.28	9.45	5.72
			2.3	101.59	63.52	38.02	22.49	15.57	10.82	6.45
		Object-to-Mouth	1	0.028	0.017	0.010	0.006	0.004	0.003	0.002
			2	0.056	0.035	0.021	0.012	0.008	0.006	0.004
			2.3	0.065	0.041	0.024	0.014	0.010	0.007	0.004
		Hand-to-Mouth	1	0.898	0.555	0.325	0.189	0.128	0.097	0.064
			2	1.819	1.132	0.672	0.396	0.276	0.197	0.119
			2.3	2.113	1.322	0.791	0.468	0.324	0.225	0.134
		Soil Ingestion	1	0.007	0.004	0.002	0.001	0.001	0.001	0.000
			2	0.014	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

Table 26. Estimated Doses via Dermal and Inhalation for Females (13-49 Years Old) at Various Distances from a Treated Field 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)[b]**	**50 (feet)**	**100 (feet)**	**250 (feet)**	**500 (feet)**	**1000 (feet)**
AT802A	2[a]	1	24.1912	20.6292	16.2365	10.9376	5.7017	3.3983	1.8125
		2	48.5586	41.2836	32.3724	21.6235	10.8244	5.8905	2.3159
		2.3	55.8424	47.4472	37.1704	24.7802	12.3901	6.5714	2.5475
	15[b]	1	22.8193	19.3076	15.0031	9.9181	5.1605	3.3480	2.4292
		2	46.7965	39.7732	31.2144	20.9691	10.9502	7.1239	5.1101
		2.3	54.4239	46.2892	36.3019	24.4039	12.9112	8.3373	5.9345
1-Hour Air Concentration at Various Distance Downwind from the Treated Fields (mg/m^3)									
			10 (feet)	25 (feet)[b]	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	0.032	0.0259	0.0174	0.0111	0.0052
		2.3	0.0428	0.0393	0.0341	0.0275	0.0183	0.0115	0.0054
	15	1	0.0403	0.0383	0.0353	0.0314	0.0257	0.0213	0.0163
		2	0.0571	0.0541	0.0495	0.0436	0.035	0.0283	0.0206
		2.3	0.0725	0.0686	0.0624	0.0546	0.0431	0.0343	0.0239

[a] Minimum spray volume as specified on some CPF product labels for the aerial application.
[b] Spray volume of 15 GPA is used for ground-based equipment and is chosen in exercise for illustrative purpose.

Table 27. Estimated Doses via Dermal for Females (13-49 Years Old) at Various Distances from a Treated Field with Chlorpyrifos using Ground-Based Equipment: Groundboom 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)
Groundboom									
High boom	40 (50^{th})[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 (90^{th})[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 (50^{th})[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 (90^{th})[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	2.65071	1.300182	0.73882	0.312144	0.164883	0.0994331
		2	13.933	5.30142	2.600364	1.47765	0.624289	0.329765	0.198866
		4	27.866	10.602839	5.200728	2.95530	1.248577	0.659531	0.397732
		6	41.799	15.904259	7.801092	4.43295	1.872866	0.989296	0.596598
Sparse Orchard	60	1	5.64880	2.572674	1.444926	0.92258	0.469475	0.283195	0.190056
		2	11.2976	5.145347	2.889853	1.84517	0.93895	0.566391	0.380111
		4	22.5952	10.290695	5.779705	3.69035	1.877901	1.132782	0.760223
		6	33.8928	15.436042	8.669558	5.53552	2.816851	1.699173	1.140334

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

Table 28. Estimated Doses via Dermal and Mouthing for Children (1-2 Years Old) at Various Distances from a Field with Chlorpyrifos using Groundboom Equipment

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	0.0131
			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	0.0392
		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-Drift deposition estimates were derived using a 50^{th} percentile or 90^{th} percentile horizontal deposition.

Table 29. Estimated Doses via Dermal and Mouthing for Children (1-2 Years Old) at Various Distances from a Field with Chlorpyrifos using Groundboom Equipment

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	0.0131	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	0.00004	0.00003	0.00003
			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	0.0653	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	0.1958	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-Drift deposition estimates were derived using a 50th percentile or 90th percentile horizontal deposition.

Table 30. Estimated Doses via Dermal and Mouthing for Children (1-2 Years Old) at Various Distances from a Treated Field with Chlorpyrifos in Apple Orchards

Scenarios	Swaths	Exposure Route	Appl. Rate (lb/acre)	Dose at Various Distance Downwind from the Treated Fields (□g/kg/day)						
				25 (feet)	50 (feet)	75 (feet)	100 (feet)	150 (feet)	200 (feet)	250 (feet)
Dormant Apple	60	Dermal	1	10.21174	3.88544	1.90582	1.08298	0.45755	0.24169	0.14575
			2	20.42348	7.77088	3.81165	2.16596	0.91509	0.48337	0.29150
			4	40.84696	15.54177	7.62329	4.33192	1.83018	0.96675	0.58300
			6	61.27043	23.31265	11.43493	6.49788	2.74527	1.45012	0.87450
		Object-to-Mouth	1	0.00652	0.00248	0.00122	0.00069	0.00029	0.00015	0.00009
			2	0.01304	0.00496	0.00243	0.00138	0.00058	0.00031	0.00019
			4	0.02609	0.00993	0.00487	0.00277	0.00117	0.00062	0.00037
			6	0.03913	0.01489	0.00730	0.00415	0.00175	0.00093	0.00056
		Hand-to-Mouth	1	0.21245	0.08083	0.03965	0.02253	0.00952	0.00503	0.00303
			2	0.42489	0.16167	0.07930	0.04506	0.01904	0.01006	0.00606
			4	0.84979	0.32333	0.15860	0.09012	0.03808	0.02011	0.01213
			6	1.27468	0.48500	0.23789	0.13518	0.05711	0.03017	0.01819
		Soil Ingestion	1	0.00159	0.00060	0.00030	0.00017	0.00007	0.00004	0.00002
			2	0.00317	0.00121	0.00059	0.00034	0.00014	0.00008	0.00005
			4	0.00634	0.00241	0.00118	0.00067	0.00028	0.00015	0.00009
			6	0.00951	0.00362	0.00178	0.00101	0.00043	0.00023	0.00014
Sparse Orchard	60	Dermal	1	8.28009	3.77106	2.11799	1.35234	0.68816	0.41511	0.27859
			2	16.56018	7.54211	4.23598	2.70468	1.37633	0.83022	0.55717
			4	33.12035	15.08422	8.47196	5.40936	2.75265	1.66045	1.11434
			6	49.68053	22.62634	12.70794	8.11404	4.12897	2.49067	1.67152
		Object-to-Mouth	1	0.00529	0.00241	0.00135	0.00086	0.00044	0.00027	0.00018
			2	0.01058	0.00482	0.00271	0.00173	0.00088	0.00053	0.00036
			4	0.02115	0.00963	0.00541	0.00345	0.00176	0.00106	0.00071
			6	0.03173	0.01445	0.00812	0.00518	0.00264	0.00159	0.00107
		Hand-to-Mouth	1	0.17226	0.07845	0.04406	0.02813	0.01432	0.00864	0.00580
			2	0.34452	0.15691	0.08813	0.05627	0.02863	0.01727	0.01159
			4	0.68904	0.31381	0.17625	0.11254	0.05727	0.03454	0.02318
			6	1.03356	0.47072	0.26438	0.16881	0.08590	0.05182	0.03477
		Soil Ingestion	1	0.00129	0.00059	0.00033	0.00021	0.00011	0.00006	0.00004
			2	0.00257	0.00117	0.00066	0.00042	0.00021	0.00013	0.00009
			4	0.00514	0.00234	0.00132	0.00084	0.00043	0.00026	0.00017
			6	0.00771	0.00351	0.00197	0.00126	0.00064	0.00039	0.00026

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

A detailed description of the CPF dietary (food only) and drinking water (DW: refined, groundwater and surface water) risk assessment for California is in APPENDIX 2. They are briefly presented below. 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). Exposures for each of these sentinel populations are summarized in Table 30 and Table 31. The PoDs for these subgroups were presented in the U.S. EPA (2014a) IRED for CPF, and in the Hazard Identification, above.

IV.B.1. Food-Only Exposure Assessment (detailed in APPENDIX 2)

IV.B.1.a. Summary of the 2014 U.S. 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 U.S. EPA risk assessment: infants (< 1 year old), children 1-2 years, children 6-12 years, and females 13-49 years (U.S. 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 (USEPA 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 U.S. 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 U.S. 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 (USEPA 2014b).

I.A.3. Residue Data and Refinements

Chlorpyrifos 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 (2009-2013), the top ten agricultural uses in the state were almond, citrus, alfalfa, walnut, cotton, grapes, corn, broccoli, sugar beet, and peach/nectarine. Average annual use for all sites, including all agricultural and non-agricultural uses, was 1.3 million lbs/year (APPENDIX 2).

U.S. 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. Chlorpyrifos-oxon residues are not included in the tolerances established for CPF residues because it is generally not found in food.

U.S. 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. Chlorpyrifos in not registered for use on potatoes in the U.S. {U.S. EPA, 2014b #654}.

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 it was 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, U.S. 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 U.S. 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.

Exposure estimates were compared to population-adjusted doses (PADs) in the U.S. EPA evaluation. PADs were based on points of departure that were estimated from physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) modeling of RBC cholinesterase inhibition in humans.

IV.B.1.b. Results of Dietary (food-only) Exposure Assessment

Exposure estimates from the 2014 U.S. EPA assessment are shown in Table 31and Table 32. Children 1-2 years old were identified as the highest exposed population subgroup: at the 99.9th percentile, exposure was 0.000423 mg/kg.

Although a commodity contribution analysis was not included in either the 2011 or 2014 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 31 Acute Dietary (food only) Exposure for CPF (U.S. EPA 2014a)

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

Table 32 Steady-state (21-day) Dietary (food only) Exposure for CPF (U.S. EPA 2014a)

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

IV.B.2. HHAB Drinking Water Assessment (APPENDIX 2)

IV.B.2.a. Summary of U.S. EPA Drinking Water Assessments

U.S. EPA conducted a preliminary drinking water assessment (DWA) in 2011 and updated it with additional analyses in 2014 (USEPA 2011a, 2014c). Chlorpyrifos is rapidly oxidized to the oxon during the chlorination process of drinking-water treatment. Since more than 75% of community water systems in the U.S. use chlorination to disinfect drinking water, the DWA assessment 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 groundwater were not expected to exceed the DWLOC. The updated DWA examined water monitoring programs across the country, including CDPR's program, and found that none of them (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 groundwater (i.e., wells) is predicted[1] 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, 21-day average concentration of CPF-oxon in drinking water derived from groundwater is not

[1] **For drinking water derived from groundwater, 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 Groundwater (SCI-GROW) version 2.3, and Pesticide Root Zone Model for GroundWater (PRZM-GM). A previous evaluation by U.S. EPA showed that, "In a few cases PRZM-GM underestimated pesticide concentration observed in groundwater", 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.

expected to be greater than 0.15 µg / L {U.S. EPA, 2014c #655}. That is less than 4% of the Drinking Water Level of Concern (DWLOC) of 3.9 µg / L for CPF-oxon[2].

- **Drinking water derived from <u>surface</u> water is predicted[3] to pose an exposure concern (Table 1, and Figure 3).** "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" {U.S. EPA, 2014c #655}. Uses that may exceed the DWLOC include scenarios for certain California cropping systems, e.g. 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[4].

- **Routine treatment of drinking water is <u>not</u> expected to mitigate the risk.** The following quotes are from {U.S. EPA, 2014c #655@@author-year}. "In general, drinking water treatment processes, with the exception of activated carbon, have been shown to have little impact on removal of pesticide residues." "It is possible that some drinking water treatment procedures, such as granular activated carbon filtration and water softening (increased rate of CPF-oxon hydrolysis at pH > 9) could reduce the amount of CPF-oxon in finished drinking water; however, these treatment methods are not typical practices across the country." "All the CPF that enters a drinking water treatment facility is assumed to be converted to CPF-oxon during treatment [chlorination]. Although CPF-oxon has a hydrolysis half-

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[2] **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 U.S. EPA's 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 {U.S. EPA, 2014c #655@@author-year}, the value 217 ppb corresponds to infants, the most susceptible population; see U.S. EPA 2014 chlorpyrifos risk assessment {U.S. EPA, 2014b #654}Table 4.8.4. The 50x "safety factors" used by Bohaty {U.S. EPA, 2014c #655} comprise 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: U.S. EPA 2014 chlorpyrifos risk assessment {U.S. EPA, 2014b #654}, p. 8.

[3] **For drinking water derived from <u>surface</u> 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 GroundWater 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." Quote is from {U.S. EPA, 2014c #655@@author-year} p. 14.

[4] **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" {U.S. EPA, 2014c #655} pp. 7-8).

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)."

IV.B.2.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, RAS 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.2.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 groundwater. A total of 1,835 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 33). 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 34).

Table 33. PDP Monitoring Data for CPF and CPF-oxon in Groundwater, Untreated Drinking Water, Finished (treated) 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
	CPF	Untreated	9	0	11
	CPF-oxon	Finished	9	0	510
	CPF-oxon	Untreated	9	0	510

YEAR	CHEMICAL	SAMPLE TYPE	SAMPLES	DETECTS	LOD (PPT)
2007	CPF	Groundwater	4	0	30
2008	CPF	Groundwater	2	0	30
2009	CPF	Groundwater	13	0	30
2010	CPF	Groundwater	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	Groundwater	8	0	30
	CPF-oxon	Groundwater	8	0	12

LOD = limit of detection.

Table 34. DEEM-FCID (v. 3.18) Acute Exposure Estimates for Chlorpyrifos Oxon in Drinking Water Based on 2001-2013 PDP Residue Data for Chlorpyrifos Oxon in Treated (Finished) Water[a]

Probabilistic Estimate With All Non-Detects at the LOD[b]			
Population Subgroup	Exposure (mg/kg/day)[c]		
	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.2.d. Analysis of Drinking Water Exposure Using EMON Surface Water Residue Data

The Environmental Monitoring Branch (EMON) at CDPR collects residue data from sampling of surface water within California by a number of government agencies including USGS, State Water Resources Control Board, and CALFED Bay-Delta Program, as well as sampling by CDPR. 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 7,154 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 35) {CDPR, 2015b #769}

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 7,048 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 36). These exposures were up to 4-fold higher than the exposures estimated based on the PDP monitoring data.

Table 35. Summary of CDPR 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 36. DEEM-FCID (v. 3.18) Acute Exposure Estimates for CPF-oxon in Drinking Water Based on 2005-2014 Surface Water Residue Data[a]

Probabilistic Estimate With All Non-Detects at the Detection Limit[b]			
Population Subgroup	Exposure (mg/kg/day)[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).

I.B.2.e. Analysis of Drinking Water Exposure Using CDPR Ground Water Residue Data

The EMON branch at CDPR collects residue data from sampling of groundwater within California by a number of government agencies including U.S. Geological Survey, CA State Water Resources Control Board, CA Department of Water Resources, CA Department of Public Health, as well as sampling by CDPR. The samples are collected from a variety of wells including municipal, community, domestic and irrigation. A total of 2,055 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 1,903 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 37) {CDPR, 2015c #770}.

Exposure to CPF-oxon in drinking water was estimated by conducting a probabilistic analysis using either the detected CPF residue in groundwater 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 2,055 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 38).

Table 37. Summary of Groundwater 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 38. DEEM-FCID (v. 3.18) Acute Exposure Estimates for CPF-Oxon in Drinking Water Based on 2004-2013 Groundwater Residue Data[a]

Probabilistic Estimate With All Non-Detects at the Detection Limit[b]			
Population Subgroup	Exposure (mg/kg/day)[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, two detections (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 (MOE and Risk Calculations)

The critical NOELs or toxicological points of departure (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 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 10 for potential neurodevelopmental effects.

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

In the assessment of single route 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 NOEL to the estimated human exposure levels. The calculation is shown below:

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

V.B. Non-Occupational Spray-Drift Bystander 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., Margin-of-Exposure [MOE]) of different exposure routes associated with spray drift were evaluated: exposures through dermal contact and inhalation for females of 13-49 years old and children of 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 of 13-49 years old, under the current buffer zone requirement of 25 feet, no risk estimates of concern were identified. 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 a.i./acre (Table 34) and groundboom and airblast at application rates of 1, 2, 4, or 6 lb a.i./acre (Table 35).

For children of 1-2 years old, risk estimates are of concern for exposures from hand-to-mouth and inhalation routes at the lowest application rate of 1 lb a.i./acre at 50 feet away from the edge of a treated field via aerial application (Table 41). 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. No risks of concern were identified for children as close as 25 feet downwind of a groundboom application, even at the highest allowed rate of 6 lb a.i./acre (Table 42). A risk of concern occurs for 1-2 year-old children 25 feet downwind of an airblast application at the rate of 6 lb a.i./acre, due to hand-to-mouth exposure (Table 44).

Table 39 MOEs for Females (13-49 Years Old) Associated with Spray Drift at Various Distances from a Treated Field 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	224	336	535	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	286	465	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	237	384	641	1230
		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	1111

Table 40 MOEs for Females (13-49 Years Old) Associated with Spray Drift at Various Distances from a Treated Field with CPF using Ground-based Equipment: Groundboom 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)
Groundboom										
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
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
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
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
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
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

Table 41 MOEs for Children (1-2 Years Old) Associated with Spray Drift at Various Distances from a Treated Field 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	316
Bell 205	2	Dermal	1	2965	4686	7652	12589	19720	33227	68006
			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	343

Table 42 MOEs for Children (1-2 Years Old) Associated with Spray Drift at Various Distances from a Treated Field with CPF using Groundboom

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
High boom	40 (90^{th})	Dermal	1	53901	75017	97022	121278	161704	202130	242555
			2	26951	37509	48511	60639	80852	101065	121278
			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

Table 43 MOEs for Children (1-2 Years Old) Associated with Spray Drift at Various Distances from a Treated Field with CPF using Lowboom Groundboom

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)
		Dermal		145533	214019	279872	363833	485111	606389	727666
				72767	107010	139936	181917	242555	303194	363833
			1	36383	53505	69968	90958	121278	151597	181917
			2	24256	35670	46645	60639	80852	101065	121278
			4	145533	214019	279872	363833	485111	606389	727666
			6	72767	107010	139936	181917	242555	303194	363833
		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
Low boom	40 (90^{th})	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

Table 44 MOEs for Children (1-2 Years Old) Associated with Spray Drift at Various Distances from a Treated Field 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-Mouth	1	475	1249	2547	4483	10611	20087	33309
			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
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

V.C. Comparison of Spray Drift Exposure Assessment modeling for CPF with the U.S. EPA

Both the U.S. EPA and this exposure assessment produced the horizontal deposition and air concentration estimates of CPF 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 Barry (2015).

V.C.1. Orchard Airblast and Groundboom

For orchard airblast and groundboom 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 identical results to AgDRIFT 2.1.1 public version. After our analysis was finished, we were able to obtain 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 expected because the empirical models that produce the orchard air blast and groundboom 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

U.S. EPA and this exposure assessment for orchard airblast simulations inputs are consistent. The only differences are due to U.S. EPA rounding up to 2 decimal places for the horizontal deposition. U.S. 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 48.

Table 45 Comparison of 50th Percentile Sparse Orchard Horizontal Deposition (pounds per active ingredient per acre [lb a.i./ac]) Across a 50 ft Wide Lawn for 20 Rows and 2 lb a.i./ac Application Rate as Estimated using the AgDRIFT Model

Distance Downwind (ft)	This Exposure Assessment	U.S. 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 (Personal Communication: Charles Peck, U.S.EPA, 2014).

V.C.1.b. Groundboom

There are no differences between U.S. EPA and this risk assessment for ground boom simulation inputs. Both used the same American Society of Agricultural Engineers (ASAE) Fine to Medium/Coarse droplet spectra for low and high boom applications. However, U.S. 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 49 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 46 Comparison of Groundboom Horizontal Deposition (lb a.i./ac) Across a 50 ft Wide Lawn for 20 Swaths and 2 lb a.i./ac Application Rate as Estimated using the AgDRIFT Model

Distance Downwind (ft)	Low Boom[a] 50th Percentile	Low Boom 90th Percentile (U.S. EPA)	High Boom[b] 50th Percentile	High Boom 90th Percentile (U.S. 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 (Per. Comm. Charles Peck, U.S.EPA, 2014).

V.D.2. Aerial Application

There are differences between U.S.EPA and this exposure assessment for aerial simulation inputs. Thus, the horizontal deposition and air concentration estimates differ between U.S.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 U.S.EPA used AgDRIFT 2.1.1 regulatory version. The U.S. EPA document (Dawson et al. 2012) on Page 14 seems to state that the AgDRIFT Tier I aerial model is a regression (empirical) model. However, the aerial Tier I AgDRIFT model is the same first principles model as Tier II and Tier III, and for Tier I aerial simulations, all inputs are assigned Spray Drift Task Force (SDTF) defaults. Those Tier I aerial default values are shown in the AgDRIFT user's manual (Teske et al. 2002b). For this comparison, the U.S. EPA Tier II modeling inputs will be compared. Table 50 shows the input comparisons for the fixed wing aircraft scenario. Table 51 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 U.S. EPA inputs, which were not changed by U.S. EPA from the defaults.

Table 47 Details of Aerial Application Inputs for AgDRIFT and AGDISP used by U.S.EPA and this Exposure Assessment

Parameters	CDPR AGDISP	U.S.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 U.S. 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- U.S. 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.

Deposition estimates for 2 lb ai/ac application rate are compared in Table 51 and shown in Figure 12. U.S. EPA AgDRIFT estimates were extended to 1000 ft downwind for comparison to CDPR AGDISP estimates. In addition, the U.S. 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. The horizontal deposition estimates of this exposure assessment are also higher than U.S. 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 California specific. In addition, U.S. 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 13). 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. Figure 12 illustrates that 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 2015).

Table 48 Comparison of Aerial Horizontal Deposition (Fraction of Application Rate) Across a 50 ft Wide Lawn for 2 lb/a.i./ac Application Rate as Estimated using the AgDRIFT and AGDISP Models

Downwind Distance (ft)	U.S.EPA AgDRIFT 2 gal/ac 20 swath AT401 Tier I	U.S.EPA AgDRIFT 2 gal/ac 20 swath AT401 Tier II	U.S.EPA AGDISP 2 gal/ac 20 swath AT401	CDPR AGDISP 2 gal/ac 50 swath AT802A	CDPR 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.

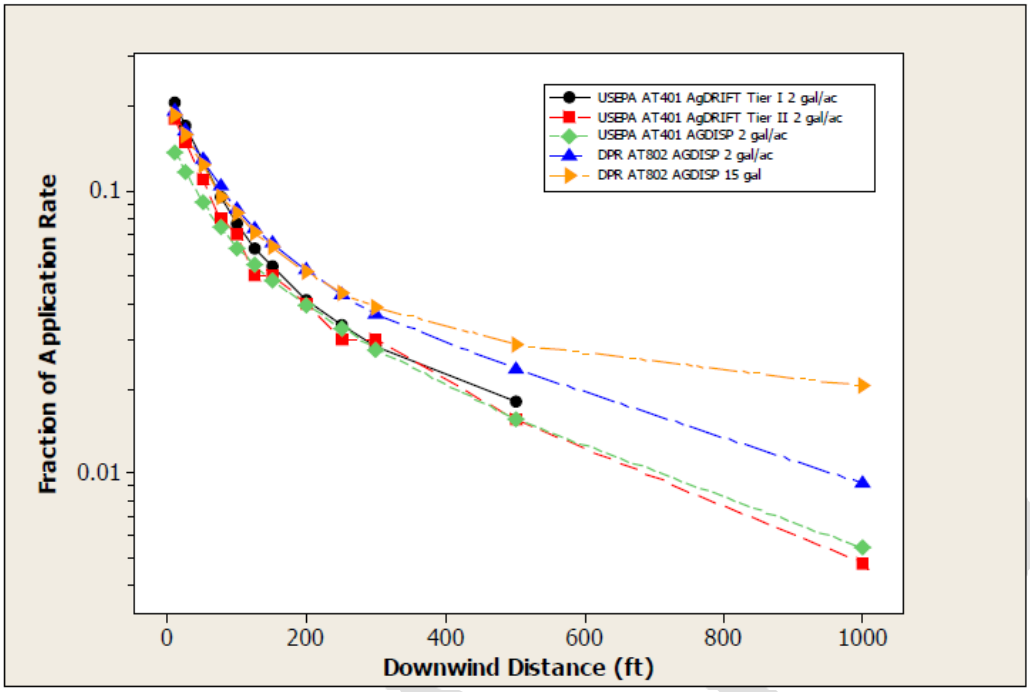

Figure 12 Aerial Application Horizontal Deposition Estimates Expressed as Fraction of 2 lb a.i./ac Application Rate as Modeled by 5 Different AgDRIFT and AGDISP Scenarios

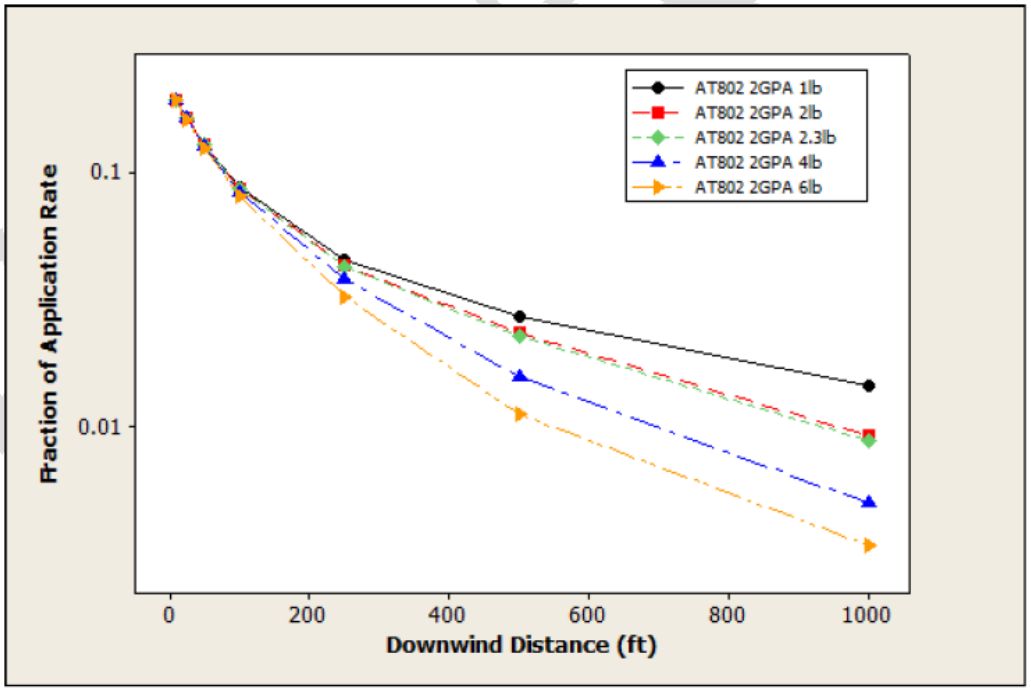

Figure 13 Effect of Application Rate on Aerial Application Downwind Horizontal Deposition Expresses as a Fraction of Application Rate.

The AT802A aircraft was used for these simulations. The simulation inputs are shown in Table 51

V.E. Dietary Risk Characterization

Dietary risk is characterized by the Margins of Exposure (MOE 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 52.

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. 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 49 Acute and Steady-state Dietary (food only) Exposure and Margins of Exposure for CPF {U.S. EPA, 2014a #383}

ACUTE DIETARY EXPOSURE[a]							
Population Subgroup	aPoD[b, c] (mg/kg)	95th Percentile		99th Percentile		99.9th Percentile	
		Exposure (mg/kg/day)	MOE[d]	Exposure (mg/kg/day)	MOE[d]	Exposure (mg/kg/day)	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/day)	MOE[d]	Exposure (mg/kg/day)	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 U.S. EPA (2014a) dietary exposure assessment to support registration review(U.S. 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 53 the CPF-oxon (ppb), water concentration (L) and body weights, obtained in the U.S. EPA (2014a) IRED 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). 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 50). 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 50 Acute CPF to CPF-Oxon Conversion for Drinking Water Residue Assessment

Population Subgroup	CPF-oxon PoD in 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 U.S. EPA (2014a) and Kwok: APPENDIX 3.
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 51shows 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 CDPR 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 51. However, lack of residue data precludes a steady-state drinking water assessment at this time.

Table 51. Acute Exposure Estimates and MOEs for CPF-oxon in Drinking Water; Surface and Groundwater

Acute Exposure Estimates for Chlorpyrifos Oxon in Drinking Water Based on 2001-2013 PDP Residue Data						
Population Subgroup	Exposure (mg/kg/day)[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 Chlorpyrifos Oxon in Drinking Water Based on 2005-2014 Surface Water Residue Data						
Population Subgroup	Exposure (mg/kg/day)[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 Chlorpyrifos Oxon in Drinking Water Based on 2004-2013 Groundwater Residue Data						
Population Subgroup	Exposure (mg/kg/day)[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. Tolerance Assessment

In California, U.S.EPA established tolerances are evaluated under the mandate of Assembly Bill 2161, generally referred to as the Food Safety Act (Bronzan and Jones 1989). The Act requires HHAB to conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides. When the risk is considered deleterious to human health, CDPR can promulgate regulations to mitigate the exposure.

The tolerance assessment is conducted for a single individual label-approved commodity (CDPR 2009; NHANES 2003-2008). The commodities are selected with potential for high exposures based on commodity contribution analyses. Exposures are presented at the 95th percentile exposure to the individual commodity with the residue level set at the tolerance.

V.F.1. Acute Dietary Exposure

For CPF, tolerances for the following commodities were evaluated: apple, banana, bell pepper, broccoli, cabbage, sweet corn, grapefruit, onion (bulb), orange, and strawberry (Table 54). These commodities were selected because of high consumption rates or high contribution to exposure in the U.S. EPA (2011a) preliminary dietary exposure assessment. MOEs were evaluated for the four sentinel populations.

The commodities with the least dietary exposure at tolerance were apple, bell pepper, sweet corn, onion, and strawberry (NHANES 2003-2008). These exposures resulted in MOEs higher than the target of

100 for all four populations. The MOEs lower than the target of 100 for one or more population subgroups exposed to a tolerance level of CPF on banana, broccoli, cabbage, grapefruit, and orange (Table 52).

V.F.2. Chronic Dietary Exposure

A chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities was not conducted because it is highly improbable, that an individual would habitually consume single or multiple commodities with pesticide residues at the tolerance levels (Table 52Table 57).

Table 52. Acute Margins of Exposure at the 95th Percentile Consumption Rate for Single Commodities with Tolerance Level Residues[a]

Commodity[b]	Tolerance	Parameters	All Infants	Children 1-2 y	Children 6-12 yr	Females13-49 yr
		Acute PoD (mg/kg)	0.600	0.581	0.530	0.469
Apple	0.01	*consumption*	0.037647	0.043133	0.013620	0.005565
		MOE	1,594	1,347	3,891	8,428
Banana	0.1	*consumption*	0.015830	0.011633	0.004687	0.002329
		MOE	379	499	1,131	2,014
Pepper, bell	1.0	*consumption (95th %-tile)*	0.000373	0.001268	0.000936	0.000759
		MOE	1,609	458	566	618
Broccoli	1.0	*consumption*	0.007739	0.009570	0.005523	0.002790
		MOE	78	61	96	168
Cabbage	1.0	*consumption*	0.003146	0.006809	0.006474	0.002469
		MOE	191	85	82	190
Corn, sweet	0.05	*consumption*	0.005068	0.007179	0.005702	0.002446
		MOE	2,368	1,619	1,859	3,835
Grapefruit	1.0	*consumption*	0.004688	0.003571	0.000552	0.003726
		MOE	128	163	960	126
Onion, bulb	0.5	*consumption*	0.001012	0.001352	0.000889	0.000658
		MOE	1,186	859	1,192	1,426
Orange	1.0	*consumption*	0.013526	0.030216	0.013319	0.009278
		MOE	44	19	40	51
Strawberry	0.2	*consumption*	0.002774	0.005247	0.002513	0.001585
		MOE	1,081	554	1,055	1,479

a- MOE = acutePoD/(tolerance x consumption). Target MOE = 100 for every subpopulation. **Shaded cells** indicate MOEs less than target, e.g., the tolerance is not health-protective at the 95th percentile consumption rate.

b- Commodities selected from 21CFR101.44 (2012); "Most frequently consumed raw fruits, vegetables, and fish in the United States" 95th percentile consumption rates (kg/kg) from DEEM-FCID, v. 3.1 (NHANES 2003-2008) and include all food forms (fresh, dried, juice, etc.).

V.G. 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 presented (Table 29, Table 30, Table 31, Table 32), 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 47) and drinking water (CPF-oxon PoD = 0.159 mg/kg/d Table 37; DW-PDP or DW-EMON).

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

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 Exposure Assessment Document (APPENDIX 3).

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

Table 50 has the CPF to CPF-oxon conversion values used in the aggregate risk characterizations for spray-drift bystander exposure. Table 53 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 53 Dermal and Oral MOEs for Children (1-2 years old) at Various Distances Downwind from Fields Treated with CPF by Aircraft or Helicopter[a]

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	125	147	186	276	530	890	1668
			2	62	73	93	140	279	513	1306
			2.3	54	64	81	122	244	460	1187
		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	251
		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	212
		CD + I + D + DW-PDP[c]	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[c]	1	43	48	55	68	95	127	193
			2	25	28	32	42	65	98	178
			2.3	22	25	30	39	61	94	171
Bell 205 Helicopter	2	CD[b]	1	98	155	253	416	651	1097	2245
			2	49	76	124	199	360	702	1602
			2.3	42	66	108	173	318	633	1471
		CD + I[c]	1	37	49	65	86	126	192	347
			2	20	27	37	51	85	145	287
			2.3	18	25	34	48	80	140	279
		CD + I + D[c]	1	36	47	62	81	115	169	277
			2	19	26	36	49	80	131	238
			2.3	18	24	33	46	76	127	232
		CD + I + D + DW-PDP[c]	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[c]	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[b]	1	133	157	202	305	586	903	1245
			2	65	76	97	144	276	424	592
			2.3	56	65	83	124	234	363	510
		CD + I[c]	1	33	36	40	47	61	75	98
			2	21	23	26	32	42	54	73
			2.3	17	19	21	26	35	44	63
		CD + I + D[c]	1	32	35	39	46	58	71	91
			2	21	23	25	31	41	52	70
			2.3	17	18	21	25	34	43	60
		CD + I + D + DW-PDP[c]	1	32	35	39	45	58	71	91
			2	21	23	25	31	41	51	69
			2.3	17	18	21	25	34	43	60
		CD + I + D + DW-EMON[c]	1	31	33	37	43	55	66	83
			2	20	22	25	30	39	49	65
			2.3	16	18	20	24	32	41	57
Bell 205 Helicopter	15	CD[b]	1	103	166	284	488	719	953	1430
			2	51	81	137	233	334	469	775
			2.3	44	70	117	197	285	410	687
		CD + I[c]	1	26	32	40	48	60	76	109
			2	17	21	27	33	43	56	84
			2.3	13	17	22	27	35	47	73
		CD + I + D[c]	1	25	32	38	46	57	72	101
			2	16	21	26	33	41	54	79
			2.3	13	17	21	27	34	46	69
		CD + I + D + DW-PDP[c]	1	25	32	38	46	57	72	100
			2	16	21	26	32	41	54	79
			2.3	13	17	21	27	34	46	69
		CD + I + D + DW-EMON[c]	1	25	31	37	44	54	67	91
			2	16	20	26	31	39	51	73
			2.3	13	17	21	26	33	44	64

a- From U.S. 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 = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion

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); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON).

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

All aggregate MOEs (Table 54) for this exposure scenario are above the target of 100 for children (1-2 years old).

Table 54 Dermal and Oral MOEs for Children (1-2 years old) at Various Distances Downwind from Fields Treated with CPF by Groundboom[a]

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
Groundboom (Children 1-2 years old)										
Highboom	40 (50th percentile)	CD[b]	1	2529	3813	5112	6493	8898	11440	14132
			2	1264	1907	2556	3247	4449	5720	7066
			4	632	953	1278	1623	2225	2860	3533
			6	421	636	852	1082	1483	1907	2355
		CD + I + D + DW-PDP[c]	1	872	984	1051	1098	1150	1183	1206
			2	651	785	875	942	1020	1074	1113
			4	433	559	655	733	833	907	964
			6	324	434	523	600	704	785	850
			1	449	477	492	502	513	519	524

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
		EMON[c]	2	382	425	450	467	485	497	505
			4	295	349	383	409	438	458	472
			6	240	296	334	364	400	425	443
Lowboom	40 (50th percentile)	CD[b]	1	4805	7066	9240	12012	16016	20021	24025
			2	2402	3533	4620	6006	8008	10010	12012
			4	1201	1767	2310	3003	4004	5005	6006
			6	801	1178	1540	2002	2669	3337	4004
		CD + I + D + DW-PDP[c]	1	1038	1113	1155	1189	1218	1237	1250
			2	856	964	1029	1084	1134	1166	1189
			4	635	761	845	921	996	1047	1084
			6	504	628	716	800	887	949	996
		CD + I + D + DW-EMON[c]	1	489	505	514	520	526	529	532
			2	445	472	487	499	509	516	520
			4	376	417	441	461	480	491	499
			6	326	374	404	429	453	469	480
Highboom	40 (90th percentile)	CD[b]	1	1780	2477	3203	4004	5339	6674	8008
			2	890	1238	1602	2002	2669	3337	4004
			4	445	619	801	1001	1335	1668	2002
			6	297	413	534	667	890	1112	1335
		CD + I + D + DW-PDP[c]	1	763	865	938	996	1060	1103	1134
			2	537	645	729	800	887	949	996
			4	337	427	504	575	669	742	800
			6	246	319	385	449	537	609	669
		CD + I + D + DW-EMON[c]	1	418	447	466	480	494	503	509
			2	340	380	408	429	453	469	480
			4	247	292	326	355	388	412	429
			6	194	237	272	302	340	367	388
Lowboom	40 (90th percentile)	CD[b]	1	2826	3875	5005	6160	8284	10010	12012
			2	1413	1937	2503	3080	4142	5005	6006
			4	707	969	1251	1540	2071	2503	3003
			6	471	646	834	1027	1381	1668	2002
		CD + I + D + DW-PDP[c]	1	904	988	1047	1088	1139	1166	1189
			2	688	790	868	928	1004	1047	1084
			4	466	564	648	716	811	868	921
			6	352	439	517	583	680	742	800
		CD + I + D + DW-EMON[c]	1	457	478	491	500	510	516	520
			2	395	426	448	463	481	491	499
			4	310	351	381	404	432	448	461
			6	255	298	332	358	392	412	429

a- From U.S. 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 = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion

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); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON).

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

Both orchard airblast scenarios show that only at the highest application rates (lb/acre) with an aggregate exposure that includes surface water have MOEs below 100 (Table 55).

Table 55 Dermal and Oral MOEs for Children (1-2 years old) at Various Distances Downwind from Fields Treated with CPF by Orchard Airblast[a]

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	434	1141	2326	4093	9687	18339	30411
			2	217	570	1163	2046	4844	9170	15206
			4	109	285	581	1023	2422	4585	7603
			6	72	190	388	682	1615	3057	5069
		CD + I + D + DW-PDP[c]	1	331	618	847	1001	1162	1230	1263
			2	189	403	624	807	1040	1154	1214
			4	102	238	409	582	859	1027	1126
			6	70	169	304	455	732	926	1049
		CD + I + D + DW-EMON[c]	1	244	370	442	481	515	528	534
			2	157	281	373	431	489	513	525
			4	92	189	283	357	445	487	508
			6	65	143	229	305	408	463	492
Sparse Orchard	60	CD[b]	1	535	1175	2093	3278	6441	10678	15910
			2	268	588	1046	1639	3220	5339	7955
			4	134	294	523	819	1610	2669	3978
			6	89	196	349	546	1073	1780	2652
		CD + I + D + DW-PDP[c]	1	386	627	814	945	1097	1175	1218
			2	226	412	589	736	940	1060	1133
			4	124	244	380	511	731	887	994
			6	85	173	280	391	598	763	885
		CD + I + D + DW-EMON[c]	1	272	374	433	467	502	518	526
			2	182	285	360	410	466	494	509
			4	109	193	269	329	408	453	479
			6	78	146	215	275	363	418	452

a- From U.S. 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 = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion

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); drinking Water (CPF-oxon PoD = 0.159 mg/kg/d from DW-PDP or DW-EMON).

VI. RISK APPRAISAL

VI.A. Introduction

Studies with potential adverse effects after acute, subchronic or chronic oral, dermal or inhalation exposure in animals have focused on ChE inhibition in plasma, RBCs and brain (See Table 7, Table 8, Table 10, Table 12, Table 13). It is well-documented that AChE inhibition is the main mode-of-action (MOA) for CPF (Eaton et al. 2008). Currently ChE inhibition is the most sensitive endpoint in pregnant or non-pregnant females (rats, mice, rabbits), males (rats, mice, dogs), as well as neonatal and developing young (rats, mice, rabbits). Hence regulation based on ChE inhibition may also be protective of other effects that may or may not be related to cholinesterase effects.

Some articles such as those presented below indicate that the effects observed on non-cholinergic systems can occur at doses equal to or lower than those inhibiting ChE (Slotkin et al. 2013; Slotkin and Seidler 2012). However Reiss et al. (2015) contend that these conclusions were reached because the ChE activity measurements were performed at a time period sufficiently long after exposure as to allow ChE

activity to recover. Therefore the maximum inhibition was unknown (e.g., underestimation of ChE inhibition) (Eaton et al. 2008). Some of these studies were performed by s.c. administration of CPF pre- or postnatally to rat pups at 1-5 mg/kg/d (Eaton et al. 2008).

VI.B. Appraisal of Hazard Identification

VI.B.1. Acute Toxicity

VI.B.1.a. Acute Oral PoDs

HHAB used a PBPK-PD model (10% RBC AChE inhibition) for the oral (dietary and spray-drift bystander) PoD for CPF (469 ug/kg/d; females 13-49 years old) even though there are studies indicating that other effects may be occurring at or below that endpoint (Table 12; Hazard ID). The decision to use this PBPK-PD was made because the model is well vetted through numerous scientific evaluations (U.S. EPA 2014a; U.S. EPA and /SAP 2008, 2010, 2012) and peer reviewed publications in the open literature (Poet et al. 2014; Smith et al. 2014; Smith et al. 2011; Timchalk et al. 2002a; Timchalk et al. 2002b; Timchalk and Poet 2008; Timchalk et al. 2005; Timchalk et al. 2006). In addition, the model is based primarily on studies performed in humans, rather than animals.

VI.B.1.b. Acute, Subchronic and Chronic Dermal and Inhalation PoDs

PoDs for CPF acute, subchronic and chronic dermal and inhalation exposures were available from the PBPK-PD modeled steady-state data presented in the U.S. EPA (2014a). The decision at HHAB was made to use the steady-state PoD values for dermal and inhalation exposures for females (13-49) (Table 19). These values were acceptable due to the sequence of CPF application which involved use for a few hours every ten days. In the spray interim, the RBC or plasma ChE that was inhibited in exposed individuals had not returned to base levels. Therefore with each CPF use, the ChE, and hence AChE inhibition, accumulated and resulted in what would become a steady-state exposure scenario. For this reason the steady-state PoDs were used for each population group at risk in lieu of acute values.

VI.B.1.c. Subchronic and Chronic Oral PoDs

Separate subchronic and chronic PoDs were not specifically calculated in the PBPK-PD model reported in the current U.S. EPA (2014a) IRED. The steady-state for 10% RBC inhibition occurred over a subchronic interval (21 days) in humans and the modeled PoD (78 ug/kg/d) was considered acceptable for subchronic oral exposure. As discussed above, the PBPK-PD model was used for HHAB oral subchronic and chronic PoDs because it is well vetted through numerous sources (Poet et al. 2014; SAP 2008, 2011; Smith et al. 2014; Smith et al. 2011; Timchalk et al. 2002a; Timchalk et al. 2002b; Timchalk and Poet 2008; Timchalk et al. 2005; Timchalk et al. 2006; U.S. EPA 2014a). The lowest 21-day steady-state PoD (78 ug/kg/d; Females 13-49 years) was acceptable for both subchronic and chronic intervals because studies performed with OPs have shown that AChE inhibition reaches steady-state at 14-21 days of exposure in studies using adult animal (U.S. EPA 2002).

VI.C. PBPK-PD Model Uncertainties

The critical NOELs or toxicological point of departures (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 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 10 for potential neurodevelopmental effects.

Interspecies Uncertainty Factor: The PBPK-PD model estimated the human RBC AChE inhibition following oral, dermal and inhalation exposures. ***Therefore HHAB employed an UF of 1 for interspecies sensisitivity when setting the target MOE for CPF.***

Intraspecies Uncertainty Factor: The PBPK-PD model predicts a time-course of RBC AChE inhibition as well as ChE inhibition in other tissues based on rates of CPF-oxon formation and ChE inhibition, reactivation and regeneration after oral, dermal and inhalation exposure to CPF. This model has been thoroughly vetted by the Scientific Advisory Panel (U.S. EPA and /SAP 2012), has undergone a "third-party quality assurance assessment,"(U.S. EPA 2014a) and has been improved in recent years by new methodologies. Improvements included the incorporation of "life-stages" mentioned previously that now includes infants (6 months), children (3-year-olds), and adults (30 year olds) (Smith et al. 2014; Smith et al. 2011). There are several uncertainties related to the PBPK-PD model, however, as shown below.

- There was a lack of data for ChE changes in women during pregnancy; a time of great physiological, anatomical change and possibly including fluctuations in RBC AChE levels (U.S. EPA 2014a).

- Poet et al. (2014) did not indicate whether the life-stage aspect of the PBPK-PD model included data for age of onset for cytochrome P450-mediated CPF detoxification via the metabolic pathway for oxidative dearylation of CPF-oxon to TCPy (biomarker and urinary metabolite). Enzyme activity in this pathway is dependent on variation in human gene expression in developing fetuses and neonates/children (Mutch and Williams 2004; Tang et al. 2001). This critical aspect of development, related specifically to age, should be incorporated into the PBPK-PD model and considered for database uncertainty factors (Faustman 2015).

- Smith et al. (2011) used human plasma and liver (13-day old infant to age 75 years) obtained from autopsies to account for age-dependent inter-individual variability of PON1 and CYP enzymes which then affect AChE inhibition. The samples were as follows: Microsomes: 30 hepatic microsome samples (13-d to 6-mos: n=7, 6-mos to 2-yr: n =6; 2 to 12-yr: n=7; 17 to 75 yr; n=10). Adult samples were selected to match adult population distributions for the primary CPF metabolizing P450s (CYP1A2, 3A4/5, 2B6, 2C19) (Buratti et al. 2003; Foxenberg et al. 2011; Mutch and Williams 2004; Sams et al. 2004; Sams et al. 2000; Tang et al. 2001) and for distributions that include potentially sensitive subpopulations while not compromising "central tendency evaluations." Plasma: Plasma samples were obtained from 20 individuals (3-d to 6-mos: n =5; 6-mos to 2-yr: n =6; 2- to 12-yr: n =4; 16-43 yrs: n=5). Hence, predictions for plasma and hepatic PON1 and hepatic P450 metabolism of CPF to the oxon and subsequent P450 detoxification

of CPF-oxon to TCPy to account for all human age-dependent inter-individual variability were based on a very small sample size.

- The microsomal activities used in Smith et al. (2011) to establish age-related differences in sensitivity to AChE inhibitors, were obtained from human autopsied tissues. There is concern that autopsied tissues are not representative of live tissues due to unknown stages of tissue degradation/autolysis before or after death. This would impact the reliability of results concerning inter-individual variability and lead to erroneous simulations.

- Critical changes in AChE and AChE-related enzyme activities (PON1, CYP enzymes) related to genetic variability and age at time of exposure suggest their relationships to the diverse responses to CPF exposure for given populations cannot easily be modeled (Griffith et al. 2011; Mutch and Williams 2004; Tang et al. 2001). A lognormal distribution of the genetic contributions to PON1 and P450 activities was assumed in Smith et al. (2011) but it overlooks data indicating that this may not be true (Harley et al. 2011; Holland et al. 2006). For example, it is well documented that human PON1 genotypes, based on ethnicity and homo/heterozygosity are associated with neonatal enzyme activity levels that may compromise the ability to detoxify CPF-oxon and/or lead to decreased fetal growth and length of gestation (Harley et al. 2011). PON1 allele frequencies were significantly varied among African Americans, Caucasians and Caribbean Hispanics affecting PON1 activity in each group (Chen et al. 2003). Polymorphisms in the PON1 coding and promotor regions that control expression can lead to lowered PON1 activity and hence, greater risk of toxicity from CPF (Eaton et al. 2008; Jarvik et al. 2003). Numerous epidemiological studies have shown polymorphism distribution frequency of plasma PON1 activity in paired mothers and their children (newborn to 9 year) (Chen et al. 2003; Eskenazi et al. 2014; Gonzalez et al. 2012; Holland et al. 2006). It was suggested by Faustman (2015) that data from those studies could be used in a modified PBPK-PD model to make it a more "robust" and "biology-based" method for approaching human variability since "it is anchored to enzyme genotypes that are well characterized." She recommended making "assumptions about population distributions of enzyme activities for PBPK-PD modeling" based on those available data. Polymorphism distributions for large, multi-ethnic populations can be derived from publically available databases (NCBI: dbSNP or The Allele Frequency Database: http://alfred.med.yale.edu/alfred). These databases could provide population diversity summaries for CPF associated PON1 and cytochrome CYP polymorphisms.

- There is concern for the use of animal data (e.g., brain tissue esterase levels) in the PBPK-PD model to represent human AChE activities to adjust for lifestage-related change (Smith et al. 2014; Smith et al. 2011). The lack of critical data for genetic variability, along with changes due to development and small datasets introduces uncertainty in the ability of the model to mimic, for example, human PON1 activity throughout all life-stages.

- The PBPK-PD model for pregnancy (Poet 2015) shows 10% inhibition of RBC AChE at 0.1-1.0 mg/kg/d for oral, 10-150 mg/m^3 for inhalation and 10-150 mg/kg/d for dermal. The range indicates steady-state (low value) to acute (high value) inhibition. The oral values are above those observed in animal models where disruption of the endocannabinoid and dopaminergic system along with behavioral effects occurred at 0.5 mg/kg/d in the absence of brain ChE inhibition. This oral

exposure level is within control levels for RBC AChE activity. Therefore there remains uncertainty about effects occurring below the level of detectable RBC AChE activity in pregnant women.

- Further detailed critique of the PBPK-PD model and the selected UF is presented in U.S.EPA (2015; EPA-HQ-OPP-2008-0850).

Therefore, due to the remaining uncertainty and gathering human and animal data related to neurotoxicity during development at doses lower than those inducing the sentinel AChE inhibition, an additional UF of 10x is employed by HHAB. As data become available we will continue to evaluate and solidify HHAB position on neurotoxicity in developing fetuses and children.

VI.D. Uncertainties Factor for Neurodevelopmental Effects

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

VI.D.1. Endocannabinoid, Adenylyl Cyclase, Serotonergic (5HTergic) and the Dopaminergic Systems:

A background on the endocannabinoid, adenylyl cyclase, serotonergic (5HTergic) and the dopaminergic systems is in Figure 4.

VI.D.1.a. CPF and the Endocannabinoid System

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

CPF-related neurobehavioral effects occur that are associated with disruption of the endocannabinoid system in pre-weanling rat pups at or below doses that affect brain AChE. MAGL and

FAAH involved in cannabinoid degradation have been demonstrated (Carr et al. 2013; Carr et al. 2011; Carr et al. 2015; Carr et al. 2014). Cannabinoids are essential in neurodevelopment, and their levels in CNS are controlled by MAGL and FAAH to keep ligand concentrations at optimal levels (Anavi-Goffer and Mulder 2009; Harkany et al. 2008). CPF may induce non-cholinergic neurodevelopmental toxicity in preweanling rats by FAAH activity. SD rats, dosed with CPF PND 10 through 16 showed FAAH inhibition that was greater and for a longer duration than brain AChE at 1.0 mg/kg/d (Carr et al. 2013). This same test regimen in SD pups was repeated CPF (0.5 mg/kg/d) by Carr et al. (2014) and showed FAAH inhibition in the absence of AChE inhibition. Since effects on the endocannabinoid system can result in neurobehavioral effects (Haring et al., 2011). It was then demonstrated that at 0.5 mg/kg/d, rat pups treated with CPF by the same protocol described above indicated that FAAH suppression (hence AEA accumulation), had significant effects on neurobehavioral responses that controls an animal's reaction to its environment as well as stress and social behavior (Carr et al. 2015). These data support a potential endocannabinoid-affected pathway for neurobehavioral disruption after pre-weaning treatment in rats. However, the Carr et al. (2015) data are preliminary and have not been peer reviewed or published and therefore cannot be used as a PoD for non-cholinergic effects.

VI.D.1.b. CPF Effects on Adenylyl Cyclase, Serotonergic (5HTergic) and the Dopaminergic Systems

VI.D.2.a. Introduction to Studies by Other Non-cholinergic Mechanisms:

A possible mechanism for developmental neurotoxicity by a noncholinergic mechanism may involve CPF disruption of cell signaling through AC (Aldridge et al. 2003). One of the most potent noncholinergic effects noted for CPF is the ability to affect the phosphorylation and function of nuclear transcription factors that control cell differentiation (e.g., 5HTergic systems) and that are themselves dependent on cAMP (Crumpton et al. 2000; Garcia et al. 2001; Schuh et al. 2002). 5HT is critical to the control of neural differentiation and organization of the developing brain (CF. 1998; Whitaker-Azmitia 1991, 2001). CPF can affect 5HT via the high-affinity presynaptic 5HT transporter (5HTT) in the developing brain (Raines et al. 2001). In addition, 5HT receptor subtypes can mediate the activity of AC. Studies of the effects of CPF on the 5HT-ergic, the dopaminergic systems and AC in developing rats have been performed (Aldridge et al. 2005a; Aldridge et al. 2005b; Aldridge et al. 2003; Aldridge et al. 2004).

Serotonin & Adenylyl Cyclase--Aldridge et al. (2003): Aldridge et al. (2003) treated pregnant rats with CPF (1.0 or 5.0 mg/kg/d) by subcutaneous (s.c.) injection during GD 17-20 (neural development) and neonates on PND 1-4 or 11-14 (post-natal differentiation and synaptogenesis). The GD17–20 developmental exposure period resulted in major alterations to 5HT receptors, the pre-synaptic 5HT-transporters and 5HT-mediated signal transduction compared with the other exposure periods. Effects on these endpoints declined when animals were treated postnatally. The AC signaling cascade was affected as measured by the summation of excitatory and inhibitory signals compared to the overall effects to 5HT. Effects at 1.0 mg/kg/d occurring during a distinct gestational period, may contribute to a noncholinergic component of CPF's developmental neurotoxicity.

Serotonin & Adenylyl Cyclase--Aldridge et al. (2004): Fetal rats were exposed to CPF via s.c. injection *in utero* (GD 9-12: neural tube stage or late gestation GD 17-20) or through postnatal neuronal differentiation and synaptogenesis (PND 1-4 or 11-14) to assess whether effects observed from treatment during these time periods continued into adulthood (PND 60). Treatment (1.0 mg/kg/d) during GD17–20 resulted in greater selectivity for regions with 5HT terminal fields (↑5HT), increased 5HT cell bodies and

5HT transporter when animals were assessed at adulthood. PN1–4 exposure resulted in increases similar to those seen from treatment GD 17-20, except presynaptic 5HT transporter was down-regulated in nerve terminals (females). Despite the fact that CPF exposure on GD17–20, PN1–4, or PN11–14 affected 5HT concentrations and hence impacted AC activity, these effects did not correspond with the effects on 5HT receptors which suggested there was an additional set of effects on proteins that transduce the 5HT signal. The developmental results of CPF exposure showed three main responses ending in long term alteration of 5HT: 1) Increased 5HT receptor expression, peaking in late gestation to early postnatal periods, primarily in males; 2) Biphasic alterations in serotonin transporter (5HTT) site (e.g., promotional activity at the transporter site after gestational CPF exposure but inhibition in regions containing 5HT terminal zones when CPF exposure is shifted to the postnatal period). This ties in with CPF effects on axonogenesis and synaptogenesis shown *in vitro* (Li and Casida 1998; Song et al. 1997) which are more active postnatally than earlier developmental periods; 3) effects on 5HT variation in cell signaling are different from those affecting 5HT receptors, which is dependent on sex, and selectivity in brain region yet both share peak sensitivity in late gestation. Long-term changes occur mainly in late gestation to early postnatal periods, which in rat is comparable to the second trimester in human fetal brain development. Disruption of the serotonergic system could potentially affect mood and appitite in humans. Effects observed in early development may contribute to neurodevelopmental behavioral disruptions in adulthood at 1.0 mg/kg/d.

Serotonin—Aldridge et al. (2005a): Rats were treated with CPF (1 mg/kg/d) using the same protocol as described above on PND 1-4 and assessed in adulthood (PND 60) for neurobehavioral effects. Adult animals showed behavioral abnormalities associated with adverse changes to 5HT-signaling (e.g., males showed more time in the open arms of the elevated plus maze, consistent with decreased 5HT and depression in animal models). Rats were unable to experience pleasure as shown by a preference for water instead of chocolate milk. Developmental cognitive function was abnormal based on behavior in the 16-arm radial maze learning and memory. Sex difference in acquisition training in CPF-treated rats (male rats usually are more accurate than females) was eliminated. CPF-treated females had decreased working and reference memory errors which corresponded with that of control males. Conversely, CPF-exposed males exhibited increased errors in these parameters. After animals were trained in radial-arm acquisition they were challenged with the 5HT receptor antagonist ketanserin. The results showed a dose-response for working and reference memory errors indicating the degree of dependence on 5HT systems. The LOEL for these effects was 1.0 mg/kg/d; Brain AChE and plasma ChE are also inhibited at this LOEL.

Serotonin and Dopamine (DA)--Aldridge et al. (2005b): As described above, fetal rats were exposed to CPF via s.c. injection *in utero* (GD 9-12: neural tube stage or late gestation GD 17-20) or throughout postnatal neuronal differentiation and synaptogenesis (PND 1-4 or 11-14) to assess the long-term effects to 5HT and DA neurotransmitters in adulthood (PND 60). Basal neurotransmitter concentrations and synaptic activities (turnover) in brain areas containing 5HT and DA projections were determined PND 60. CPF treatment resulted in increased 5HT turnover on GD17–20 and PN1–4 but neurotransmitter content was not affected. Dopamine however was greatly decreased in the hippocampus at 1.0 mg/kg/d CPF during GD17–20 due to overt toxicity. Results indicate that with CPF exposure in a critical developmental period, there is lasting activation of 5HT systems in association with 5HT-associated behavioral anomalies. Exposure to CPF alters neuronal development of 5HT and DA systems as well as affecting AC at 1.0 mg/kg/d (lowest dose tested). At this dose, there is also inhibition of RBC, plasma and brain AChE (Table 7, above), so that these non-cholinergic effects cannot be separated from cholinergic effect. The Aldridge et al. studies did not co-examine AChE for comparison. In addition, all studies

administered CPF by s.c. injections which is not a representative route for human exposure. Futhermore, the doses (≥ 1.0 mg/kg/d) were higher than those showing plasma ChE inhibition in rats (Carr et al., 2014). It would have been helpful to test at lower doses and include the assessment of AChE activity.

VI.E. Uncertainties related to CPF and Plasma ChE (BuChE) During Neurodevelopment

CPF has been shown to affect plasma/BuChE during development in numerous studies described above (Table 7, Table 12, Table 13). 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 (Carr et al. 2014; Marty and Andrus 2010). Zheng et al. (2000) demonstrated greater BuChE inhibition than RBC AChE in rat neonates after both acute and repeted 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, 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. Therefore, the role of plasma/BuChE inhibition in neurodevelopment introduces uncertainty as to the long-term occurring at doses lower than those inhibiting RBC AChE.

VI.F. Uncertainties from Human Studies

VI.F.1. Columbia Cohort Study

There are methodological limitations of the CCCEH study (e.g., only a single maternal blood sample was collected; only a single air sample was collected over 2 days; low number of participants and others detailed in Reiss et al. (2015)). CCCEH also did not report an estimate of post-natal CPF exposure in the child subjects; hence it is not known how continued CPF exposure may have exacerbated *in utero* effects, or caused adverse effects related to post-natal exposure.

The MRI results prompted the U.S.EPA to request input on methodologies (Rauh et al. 2011; Rauh et al. 2006) that lead to the MRI study (U.S. EPA 2012d). Cognitive testing used in the earlier publications was appropriate, according to reviewers but they could not comment on the tests that were administered due to lack of information provided in the corresponding publications. The Weschler Intelligence Scale and Bayley Scales of Infant Development used in the studies were useful for showing that the more highly exposed (≥6.17 pg/g) children actually scored more poorly (psychomotor and mental development and full-scale IQ) than those less exposed. However, it was stated that the population evaluated in the CCCEH cohort was not directly comparable to a general population, since the children experienced a higher CPF exposure.

The generalized effect on white matter integrity (enlargement) and reduced cortical thickness in scattered areas across the brain surface in children exposed to higher levels of CPF was confirmed. Some reversal of usual female vs. male differences in sexually dimorphic brain regions (e.g. parietal lobe size) was seen in high exposure children (high CPF exposed ≥4.39 pg/g; low CPF exposed <4.39 pg/g). Morphologic changes appeared to be related to lower IQs in these children and the results were found to "support the contention that exposure to CPF, even for some in the low CPF exposure group, is related to general cognitive deficits." In addition, the results provided "convergent evidence" with the findings of a reduced thickness of the parietal cortex in rat offspring in the DNT Health Effects Test Guideline study (Hoberman 1998) submitted by Dow AgSciences. Another conclusion was that "the results of this study suggest that one might expect that the most common effects of CPF exposure would be similar to the most common effects associated with a range of developmental brain insults, effects such as attention deficits, learning disabilities and deficits in social development."

The 2008 FIFRA SAP raised the possibility that results on child mental and motor development observed in the Columbia Cohort study (Rauh et al. 2011; Rauh et al. 2015; Rauh et al. 2006) after exposure to CPF may have been confounded by co-exposure with diazinon, another AChE inhibitor. Whyatt and Rauh (2010) showed that when diazinon was added to the model, the magnitude of the statistical risk for CPF effects on Mental Development Index (MDI) and a Psychomotor Development Index (PDI) was increased (↑effect of CPF on MDI and PDI = 50-200% in the same direction "away from the null"). These results suggested that diazinon confounds the effects of CPF alone (diazinon negative correlation with CPF=0.63; Whyatt and Rauh (2010)) which can result in an underestimation of effects related to CPF.

Concerns related to the Columbia Cohort study have been addressed in the U.S. EPA (2014a) IRED; the SAP reports (U.S. EPA and /SAP 2008, 2012) and in open literature reviews (Mink et al. 2012; Reiss et al. 2015). HHAB agrees with the U.S. EPA (2014a) and the SAP (U.S. EPA and /SAP 2008, 2012) conclusion regarding this study: "CPF likely played a role in the observed neurodevelopmental outcomes" in children. However, data are still being analyzed from the cohort of children from this study and the long-term effects of CPF exposure to human fetuses and young children have been reported (Rauh et al. 2011; Rauh et al. 2015; Rauh et al. 2012).

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

Concerns have arisen about use of the empirical data of Rauh et al. (2006) and Rauh et al. (2011) and about its use in the PBPK-PD model to measure decrements in working memory at age 7 (Dow AgroSciences, personal communication: August, 2015). Concerns include the facts that 1) Exposure classifications used in their PBPK application had measurements of plasma CPF concentrations that were below the validated limit of quantitation (LOQ: no replicates); 2) the analytical lab that performed the original analyses has since increased the LOQ to 21 pg CPF/g serum; 3) exposures had a great deal of variability. Rauh et al. (2006), however, stated the "CPF exposure levels ranged from nondetectable to 63pg/g. We imputed exposure levels in participants with nondetectable CPF (n = 115, 43%) according to assay-specific LOQ values, with 93 subjects having LOQ equal to 0.5 pg/g and 22 subjects having LOQ equal to 1 pg/g." In addition, statistical methods were performed to make estimates below the LOQ which is well-documented in articles related to the Columbia Cohort.

The model originated by Hattis (2015) provides a means of examining potential exposure levels on developing human fetuses. Working memory is a measurement of brain function (ability to memorize, retain and manipulate new information). Steady state values obtained in the Rauh et al. (2006) study for working memory decrements in children at age 7 show that 6.17 pg CPF/g maternal blood (where maternal:fetal ratio of CPF values in blood ~ 1.0; range =1.065 – 1.35) is a level that approximates a NOEL (Whyatt et al., 2004). Currently, the PBPK-PD-modeled steady-state PoD generated by the U.S. EPA (2014a) for women age 13-49 (based on 10% AChE inhibition) is 0.078 mg/kg/day. However, Hattis (2015) found that by performing dose reconstruction based on human data (Kisicki et al. 1999; Nolan et al. 1984; Rauh et al. 2011; Smith et al. 2014; Smith et al. 2011), maternal exposures correlated with mental and motor delays in offspring (range 0.35 ug/kg/d for inhalation to 0.43 ug/kg/day for oral). It was suggested that there may be no threshold for loss of working memory. The data from this model are potentially useful for generating PoDs for oral or inhalation CPF exposures in humans.

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

The biomarker for CPF exposure in this study for the Mt. Sinai cohort was TCPy in urine (Berkowitz et al. 2004). At the highest level of OP exposures was there a trend between CPF-related head circumference decrease and $PON1_{192}$ RR genotype in subjects with TCPy levels greater than LOD.

VI.F.4. ToxCast and Zebrafish HTS Assays

Currently the ToxCast HTS assay results for CPF cannot be used for risk assessment because the true actives are not related to any specific MOA or AOP. Assays considered active on the ToxCast Dashboard for steroid hormones or estrogen, androgen or thyroid receptors are in the region of cytotoxicity. Lack of metabolic activity in the assays is a reason why CPF is not active with AChE where CPF-oxon is. CPF-oxon shows true activity with numerous CYP assays, which supports the known CPF metabolism but it does not reveal anything new about risk unless it can be related to a specific genotype for CPF-related CYP enzymes. The ToxPi comparisons indicated that while the Toxicity Scores are similar, the active assays were varied between CPF and CPF-oxon. This is an expected result since there are few in vitro assays that test for neurotoxicity and there is no metabolic activation for the parent compound. At this time ToxCast doesn't add new information for the CPF risk assessment.

ToxCast-related work of Padilla et al. (2012) and Truong et al. (2014) did not report that ZF neurobehavioral effects from CPF were tested. Malformations were observed with CPF and recorded as a Terata Score with the "chorion intact" method with an AC_{10} of 3.0 uM (~NOEL) and an AC_{50} of 8.5 uM (Padilla et al. 2012). No malformations occurred by the method with the "chorion removed" at concentrations as high as 64 uM CPF (Truong et al. 2014). The studies discussed below provide more information on neurobehavioral/neuromuscular and AChE inhibiton activities associated with CPF treatment to ZF.

The ZF model was supportive for evidence of the ability of animals to metabolize CPF to form toxic metabolites that ultimately lead to neurobehavioral/learning/cognition effects (unrelated to neuromuscular degeneration) Levin et al. (2003). At very low doses (0.01 ug/ml) ZF (chorion intact) showed effects on average choice accuracy, decreased spatial discrimination, increases in average latency response which persisted into adulthood when the animals were first tested (20 weeks). Richendrfer et al. (2012a) treated

ZF (chorion intact) for a "subchronic" time-period (1 through 7 dpf) with CPF. Results showed anxiety-related behaviors in ZF at ≥0.01uM including thigmotaxis (edge preference) anddecreased swim speed.

Swim activity was inhibited after CPF treatment at 0.10 uM for 5 dpf (Levin et al. 2003; Levin et al. 2004) and was considered to be associated with neuronal death due to AChE inhibition and subsequent hyperstimulation at neuromuscular junction (Behra et al. 2002b; Yen et al. 2011). Jin et al. (2015) showed both neurobehavioral and teratogenic effects after CPF treatment (0.10 uM) to embryos that persisted into adulthood (no chorion; 1-5 dpf), in addition to AChE inhibition.

Sledge et al. (2011) also showed that ZF embryos (chorion intact) treated with low doses of CPF (lowest dose tested: 0.10 ug/ml; chorion intact) had persistent declines in brain dopamine and norepinephrine levels and increased neurobehavioral effects that persisted including decreased habituation to startle, "trend toward increased overall startle response", and a lower learning rate. When introduced to a novel tank environment, the ZF showed decreased escape diving response and increased swimming.

Subsequently Richendrfer and Creton (2015) showed that AChE was significantly decreased only at 0.1 uM CPF, where at ≥0.01 uM CPF caused neurobehavioral effects after 1-5 dpf treatment. ZF treated during 3-5 dpf showed a significant decrease in percent preference for location in their swim lane at ≥0.01 uM in a complete absence of AChE inhibition. These results show that at CPF concentrations lower than those that inhibit AChE the behavior of ZF are affected during development. Window of exposure was also important. This supports the finding of Rauh et al. (2012), Whyatt and Rauh (2010), Hattis (2015), Carr et al. (2013); Carr et al. (2015); Carr et al. (2014), Mohammed et al. (2015) and others that have shown loss of CNS-related neurobehavioral effects at exposures lower than those inhibiting AChE.

The CNS-related neurobehavioral effects from CPF at doses lower than those causing AChE inhibition were also reported for rodent models (Aldridge et al. 2004; Icenogle et al. 2004; Levin et al. 2001; Slotkin and Seidler 2007). Effects included increased startle response, increased hyperactivity, and impairment in learning. These were similar to results observed in epidemiological studies (Benmoyal-Segal et al. 2005; Carr et al. 2013; Carr et al. 2015; Carr et al. 2014; Mohammed et al. 2015; Rauh et al. 2011; Rauh et al. 2015; Rauh et al. 2006; Rauh et al. 2012; Whyatt and Rauh 2010). Taken together, the ZF, rodent, and human data provide strong weight-of-evidence for the ability of CPF to cause irreversible developmental toxicity, behavior alterations, and metabolic enzyme alterations at very low doses (10x lower than those that cause AChE inhibition in ZF). Although ZF are not mammals, common genes for similar gene function (e.g., AChE) have been conserved across species (Linney et al. 2004); hence the results in this model support the hypothesis that neurobehavioral toxicity initiated in embryos is insidious and permanent at low concentrations of CPF.

These studies provide strong weight-of-evidence for the ability of CPF to cause neurodevelopmental toxicity related to learning/cognition/behavior at doses 10x lower than those that cause AChE inhibition that would lead to neuromuscular effects in ZF (0.01 vs. 0.10 uM).

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

There was no interspecies uncertainty in the CPF risk characterization since the PoDs were selected from the U.S. EPA (2014a) PBPK-PD model which is based primarily on studies performed in humans, rather than animals. Therefore the default 10x interspecies uncertainty factor (UF) was not applied.

An UF of 10 for intraspecies variability for oral, dermal and inhalation exposure was based on physiological changes (e.g., AChE fluctuations) in women during pregnancy (U.S. EPA 2014a). This intraspecies variability in the UF also pertains to male and female infants, children and youths since the data used by Smith et al. (2014) to model age-related variability (age 6 months to >16 years) used few samples (30 hepatic microsome, 20 plasma samples) to estimate intra-individual age-related variability of PON1 and cytochrome P-450 enzyme activity for all subpopulation groups (including variability representing all ethnic populations). Different ethnic populations demonstrate vastly different PON1 activities (Diepgen and Geldmacher-von Mallinkrodt 1986) and P450 phenotypes, factors that caninfluence CPF toxicity. Of the 120 parameters in the CPF PBPK-PD model only 16 were used for variability in the Data Derived Extrapolation Factor (DDEF) intra-species analysis. Only four of the 16 parameters were used to drive more than 80% of the RBC AChE inhibition (hepatic P450 metabolism of CPF → CPF-oxon, hepatic P450 detoxification of CPF-oxon → TCPy; hepatic PON1 detoxification of CPF-oxon → TCPy, plasma PON1 detoxification of CPF-oxon → TCPy) (U.S. EPA 2014a). The variations are due to genotypic and phenotypic differences which affect and the rates of detoxification and activation in humans (Berkowitz et al. 2004; Diepgen and Geldmacher-von Mallinkrodt 1986; Furlong et al. 2006). CPF was found in 70.5% of pregnant mothers living in the Salinas Valley in California (Huen et al. 2010) putting both fetuses, that cannot metabolize OP, as well as their mothers, at risk (Chen et al. 1999; Furlong et al. 2006). Of concern as well is the uncertainty that autopsied tissues used for input data may or may not produce the relevant enzyme activities (i.e. plasma PON1, hepatic PON1, hepatic P450 bioactivation to oxon and hepatic P450 detoxification to TCPy) resembling normal human microsomal or plasma enzymes, even though the PBPK-PD model is designed to compensate for their potential differences (Poet 2015; Smith et al. 2011). Various uncontrolled processes of autolysis and degradation along with inconsistent quality of tissues can ultimately affect the interpretation of data derived from them. Therefore the UF of 10 is used to account for intraspecies variability related to age, inter-and intra-ethnic differences in enzyme activities (e.g., PON1 and P450) and genotypic frequencies in populations that have greater susceptibility to CPF toxicity (Eaton et al. 2008; Jarvik et al. 2003).

A further UF of 10 is based on neurodevelopmental and neurobehavioral effects occurring in human fetuses *in utero* and during development (Hattis 2015; Horton et al. 2012; Lovasi et al. 2011; Perera et al. 2003; Rauh et al. 2011; Rauh et al. 2006; Rauh et al. 2012; Reiss et al. 2015; Whyatt et al. 2009; Whyatt et al. 2007; Whyatt et al. 2004) at exposure levels lower than those inducing RBC, plasma or brain AChE inhibition. Berkowitz et al. (2004) showed an association with PON1 status and head circumference in children exposed to CPF *in utero*. Data also support the findings of disruptions from CPF in the CNS (serotonergic and endocannabinoid pathways) at exposure levels lower than those inducing brain AChE inhibition in preweaning rats (<0.5 mg/kg/d) that result in neurobehavioral/neurodevelopmental effects (Carr et al. 2015; Carr et al. 2014; Mohammed et al. 2015).

The biological mechanism of decrements in working memory following CPF exposure has not yet been reported in animal models or in epidemiological studies. There is no projected adverse-outcome pathway showing effects of CPF on developing brain due to lack of data for cause-effect activities. Hattis' 2015 proposed model for linking potential exposure with decrements in working memory in 7 year old children that had been exposed to CPF *in utero* was preliminary and not yet peer reviewed. Uncertainty remains about the CPF exposure *in utero* and in childhood and the observed neurodevelopmental effects that are detected from birth into preadolescence (Rauh et al. 2015). Therefore, due to the remaining uncertainty and gathering human and animal data related to neurotoxicity during development at doses lower than those inducing the sentinel AChE inhibition, an additional UF of 10x is employed by HHAB (Table 56).

Therefore the target MOE for each acute or steady-state population subgroup was 100 and it applies to all the population subgroups described in this risk assessment (all infants <1 year old; children 1-2 years old, children 6-12 years old and females 13-49 years old) in dietary and bystander spray drift, including oral, dermal and inhalation exposure scenarios for children (1-2 years old) and females (13-49 years old) (Table 56).

Table 56 Summary of Uncertainty Factors for CPF Oral (diet, drinking water) and Spray Drift Exposure

Route	Duration of Exposure			
	Acute		Steady State (21 day)	
	Intraspecies Variability	Neurobehavioral/ neurodevelopmental[a]	Intraspecies Variability	Neurobehavioral/ neurodevelopmental[a]
Oral	10	10	10	10
Dermal	10	10	10	10
Inhalation	10	10	10	10

a- Neurobehavioral and neurodevelopmental effects have been indicated at doses below those inducing effects on AChE.

VI.H. Uncertainty Factors used by the U.S. EPA

The U.S.EPA uses different UF, or extrapolation factors than HHAB. It is documented in the U.S.EPA IRED that the PBPK-PD model for 10% RBC AChE inhibition in humans after CPF exposure was chosen for PoD determination for all population subgroups considered (U.S. EPA 2014a). The U.S.EPA calculated PoDs for oral (dietary, drinking water, non-dietary oral), residential, and occupational exposures by inputting into the PBPK-PD model, pertinent data based on scenario (e.g., route: oral, dermal, inhalation, aggregate), populations exposed, body weights (vary by lifestage), duration of exposure (hours/day; days/week) and frequency of exposure. The modeled PoD included a 10x "safety factor" (SF) based on the Food Quality Protection Act (U.S. EPA 1997b) and 10x intra-species extrapolation factor to give 100x for Females 13-49 who may be or may become pregnant. All other population subgroups also have the 10x FQPA SF as well as an additional 4x intra-species extrapolation SF (based on the 99th percentile for variation in sensitivity from a Data Derived Extrapolation Factor) for all other subpopulations (40x total SF/UF). For drinking water assessment, only CPF-oxon was of concern because it is assumed that all CPF in water converts to CPF-oxon after treatment. Due to this conversion U.S.EPA uses an additional 5x uncertainty factor for intra-species extrapolation (50x total SF/UF). The PoDs were divided by the total extrapolation factors for each subpopulation to obtain a Population Adjusted Dose (PAD) which is essentially a reference dose. HHAB does not calculate PADs but instead uses MOEs for risk characterization.

V.I. Acute CPF Spray Drift Exposure Appraisal

Akin to the U.S. EPA, this exposure assessment employed state-of-the-art computer models (AgDRIFT and AGDISP) coupled with the latest version of the U.S. 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 SOP were applied. The intrinsic uncertainties associated with these computer models and SOP have been detailed in the original documentations (Teske et al. 2002a; Teske and Curbishley 2013; U.S. EPA 2012). 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 give the highest drift deposition and air concentration estimates in California (Barry 2015). Hence, these exposure estimates of CPF can be considered as the realistic upper bound values anticipated in California. Unlike the aerial application, the available computer models are unable to generate the air concentration of CPF from groundboom and orchard airblast. However, the ambient air concentrations of CPF measured after a ground based application in an orange orchard in Tulare, CA (up to 0.0472 mg/m^3 at 42 feet from the edge of the field) (CARB 1998) are similar to the simulated values from an aerial application (Table 23). This comparison suggests that ground based application methods may be as important as those of aerial application in contributing to the airborne CPF at locations away from the treated field. The lack of air concentration estimates for groundboom and airblast applications leads to an underestimate of exposure and risk for bystanders to these applications.

For the drift 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 the drift 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 (Table 57) and California (Table 58) are essentially identical.

Table 57 MOEs for Children (1-2 Years Old) Associated with Spray Drift at Various Distances from a Treated Field 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	3241	3800	4829	7168	13750	23070	43257
			2	1615	1899	2422	3626	7243	13310	33853
			2.3	1404	1652	2109	3164	6328	11931	30776
		Object-to-Mouth	1	3818	4477	5688	8444	16198	27176	50955
			2	1902	2237	2853	4271	8532	15679	39878
			2.3	1654	1946	2485	3727	7454	14054	36253
		Hand-to-Mouth	1	117	137	175	259	497	834	1564
			2	58	69	88	131	262	481	1224
			2.3	51	60	76	114	229	431	1113
		Soil Ingestion	1	18347	21515	27335	40578	77842	130601	244877

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	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	316
		CD[b]	1	44	50	58	74	112	161	292
			2	24	27	33	44	71	115	255
			2.3	22	25	30	40	66	109	242
Bell 205	**2**	Dermal	1	2538	4011	6550	10777	16881	28443	58215
			2	1260	1979	3214	5165	9325	18213	41526
			2.3	1096	1720	2792	4476	8257	16414	38144
		Object-to-Mouth	1	2990	4725	7716	12695	19885	33505	68575
			2	1485	2331	3786	6084	10984	21455	48917
			2.3	1291	2026	3289	5273	9726	19335	44933
		Hand-to-Mouth	1	92	145	237	390	610	1029	2105
			2	46	72	116	187	337	659	1502
			2.3	40	62	101	162	299	594	1379
		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	343
		CD[b]	1	35	46	62	83	122	187	338
			2	18	25	35	49	82	141	279
			2.3	17	23	32	46	77	135	271

a Risk estimates generated using TTR data from Mississippi (Stafford and Robb 1999)
b-CD = Combined Deposition (Aggregated MOEs for all routes: Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion).

Table 58 Aggregate MOEs for Children 1-2 years at Various Distances Downwind from Fields Treated with CPF by Aircraft or Helicopter[a]

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)										
		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	251
	2	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	212
		CD + I + D + DW-PDP[c]	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[c]	1	43	48	55	68	95	127	193
			2	25	28	32	42	65	98	178
1			2.3	22	25	30	39	61	94	171
Bell 205 Helicopter	2	CD[b]	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[c]	1	37	49	65	86	126	192	347
			2	20	27	37	51	85	145	287
			2.3	18	25	34	48	80	140	279
		CD + I + D[c]	1	36	47	62	81	115	169	277
			2	19	26	36	49	80	131	238
			2.3	18	24	33	46	76	127	232
		CD + I + D + DW-PDP[c]	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[c]	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[b]	1	135	160	205	311	597	921	1269
			2	66	78	99	147	282	433	603
			2.3	57	67	85	126	239	370	519
		CD + I[c]	1	33	36	40	47	61	75	98
			2	21	23	26	32	42	54	73
			2.3	17	19	21	26	35	44	63
		CD + I + D[c]	1	32	35	39	46	58	71	91
			2	21	23	25	31	41	52	70
			2.3	17	18	21	25	34	43	60
		CD + I + D + DW-PDP[c]	1	32	35	39	45	58	71	91
			2	21	23	25	31	41	51	69
			2.3	17	18	21	25	34	43	60
		CD + I + D + DW-EMON[c]	1	31	33	37	43	55	66	83
			2	20	22	25	30	39	49	65
			2.3	16	18	20	24	32	41	57
103	15	CD[b]	1	105	170	290	498	733	972	1458
			2	52	83	140	237	340	478	790
			2.3	44	71	119	201	290	418	701
		CD + I[c]	1	26	32	40	48	60	76	109
			2	17	21	27	33	43	56	84
			2.3	13	17	22	27	35	47	73
		CD + I + D[c]	1	25	32	38	46	57	72	101
			2	16	21	26	33	41	54	79
			2.3	13	17	21	27	34	46	69
		CD + I + D + DW-PDP[c]	1	25	32	38	46	57	72	100
			2	16	21	26	32	41	54	79
			2.3	13	17	21	27	34	46	69
		CD + I + D + DW-EMON[c]	1	25	31	37	44	54	67	91
			2	16	20	26	31	39	51	73
			2.3	13	17	21	26	33	44	64

a- From U.S. EPA (2014a): 21-Day steady-state PoDs: Dermal: 134.25 mg/kg/d; Oral: 0.099 mg/kg/d, Inhalation steady: 2.37 mg/m^3
b- Combined Deposition = Dermal + Object-to-Mouth + Hand-to-Mouth + Soil Ingestion
c- Combined deposition (CD = dermal + object-to-mouth + hand-to-mouth + soil deposition; inhalation (I); dietary (D: food only; acute oral PoD for CPF: 0.581 mg/kg/d); drinking water; acute PoD for CPF-oxon: 0.159 mg/kg/d from DW-PDP or DW-EMON).
Target MOE = 100

V.J.Issues Related to Food Exposure

V.J.a. Illegal Residues in Food Were Not Included in the Exposure Assessment:

The PDP data indicate that CPF residues are frequently detected on crops that lack CPF 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 CPF residues on catfish, cilantro, cherry tomatoes, green onions, spinach, and five other crops.

From 2012 to 2014, the CDPR's residue monitoring program detected illegal CPF residues on 18 commodities. A high proportion of samples of cactus (leaves or fruit), litchi, and longan contained illegal CPF residues. 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. It should be noted that the goal of CDPR's program is regulatory compliance, so samples are prepared according to the tolerance definition (usually "in or on"), while the PDP program is designed for dietary risk assessment so standard consumer practices such as rinsing are followed and only the edible portion of samples is analyzed for pesticide residues. Therefore, CDPR's monitoring may detect illegal residues more frequently or at higher concentrations than those detected by PDP.

RAS does not evaluate illegal residues on agricultural commodities in its dietary exposure assessments. However the high frequency of these detections for CPF suggests there could be additional exposures not considered in the dietary assessment.

V.J.b. Dietary Risks Evaluated on a Per Capita Basis Rather than Per User

In this risk document, RAS calculated the risk from CPF exposure from food using the 2014 U.S. EPA's exposure values, which were estimated on a per capita (all individuals surveyed) basis. RAS selects per user-day (consumers only or the population that is exposed) basis 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 CPF 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.

V.K. CPF Risk Appraisal for Drinking Water

U.S. EPA modeling of surface water residues predicted that certain CPF 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 CPF was not modeled under California-specific conditions.

RAS estimated drinking water probabilistic exposures using (1) PDP residue data for CPF-oxon in treated drinking water in California or (2) monitoring data for CPF in surface and groundwater 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 CPF or CPF-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 CDPR surface and ground water programs are designed to 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 biased toward capturing higher concentrations coinciding with runoff timing, storm events, high-use regions, and application timing. The CDPR 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 as sources for drinking water. Consequently, a drinking water exposure based on these residues would likely represent the "high-end" of the potential exposure. Regardless of the residue database, all acute drinking water MOEs at the 99.9th 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 CPF in the California drinking water is likely to be somewhere between the "high-end" exposure scenarios based on the CDPR surface and ground water detections and the scenario based on LOD for CPF-oxon from the PDP monitoring.

VII. CONCLUSIONS

The health risk assessment of CPF was carried out for 4 sentinel subgroups of the general population: infants (<1 year old), children 1-2 years, children 6-12 years, and women of childbearing age (13-49 years).

Single-route exposure scenarios were evaluated for children 1-2 years and women 13-49 years under acute conditions associated with spray drift near the application site: (i) dermal exposure through skin contact, (ii) inhalation exposure, and (iii) 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 or subchronic (21-day, steady-state) durations and drinking water acute exposures were calculated for the 4 population subgroups. Aggregate exposures involving multiple routes were also calculated for children 1-2 years staying at 10-1000 feet from the CPF application site that could be exposed through inhalation, skin contact with residues (drift deposition), ingestion of residues by object-to-mouth + hand-to-mouth + 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 characterizing the risk from exposure to CPF were PBPK-PD-estimated human equivalent doses based on 10% RBC AChE inhibition. A margin of exposure of 100 is generally 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 yrs: The MOEs for dermal and inhalation 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; groundboom and airblast at the application rates of 1, 2, 4, or 6 lb a.i./acre.

Children 1-2 yrs: All MOEs for dermal and oral exposures (object-to-mouth and incidential 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 an aerial or airblast equipment at application rate of 1 lb a.i./acre.

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 at 25 feet of the airblast application at 6 lb a.i./acre. The inhalation MOEs were lower than the target of 100 for children staying 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 an aerial equipment. Consequently, mitigation should be considered for children 1-2 years near the sites where CPF is applied with aerial equipment, and in conjunction with their potential aggregate exposures.

Dietary Exposure:

Food-only exposure: At the 99.9th percentile, the acute dietary MOEs from exposure to CPF residues in food ranged from 1,374 to 3,127 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 1,040. 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 CDPR's Environmental Monitoring Branch (EMON) surface and ground water program. At the 99.9th percentile, the MOEs were highest for PDP (1571-3970) and lowest for the CDPR surface water (405 – 1,299). All MOEs for acute water-only exposure were greater than the target of 100.

Aggregate Exposure: Dietary (food only), Drinking Water (PDP or CDPR surface water) and Spray-Drift

Children 1-2 yrs: The acute aggregate MOEs were estimated for all routes, including combined deposition. Because these exposure routes occurred in different time frames (e.g., 1.5 h for dermal, inhalation and non-dietary oral, and 1 day for dietary exposure), an aggregate MOE approach was used for evaluating the total potential exposure.

For the combined deposition, the risk was calculated using the 21-day steady state dermal, inhalation and oral PoDs for CPF and the acute (1.5 hours) dermal, inhalation, and non-dietary oral exposures (Table 60). The acute dietary risk from food-only or drinking water probabilistic 99th percentile exposures was calculated using the acute oral PoD for CPF and the acute oral PoD for CPF-oxon, respectively. The drinking water exposures were based on residues from PDP or the CDPR EMON surface water program.

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

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 CDPR's Environmental Monitoring (EMON) surface water database.

The aggregate MOEs for a number of combined scenarios were below the target of 100 (Table 60). The air exposure had a substantial contribution (up to 95%) to the aggregate exposure. Consequently, the combined 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 the application site was identified as a driver of the aggregate MOEs below the target value of 100 for children 1-2 years for the indicated scenarios in Table 60.

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APPENDIX 1 HHA BRANCH SUMMARY OF TOXICOLOGY DATA 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/day, 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/day 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/day, but a practical NOAEL for ChE inhibition is 0.1 mg/kg/Day. 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/day). 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/day 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/day, 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/day (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/day 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/day. 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/day (based on slight plasma ChE inhibition at 0.1 mg/kg/day in females). Acetylcholinesterase ChE inhibition NOAEL of 0.1 mg/kg/day 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/day [based on very slight (≤ 3%) but often statistically significant body weight decrease in 1 mg/kg/day males]. Body weights were statistically significantly reduced in 10 mg/kg/day males (7 to 9% throughout study). The "non-ChE effects" NOAEL was 1 mg/kg/day. Findings at 10 mg/kg/day 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/day 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/day in diet. NOEL cholinesterase enzyme inhibition = 0.1 mg/kg/day. NOEL for other systemic effects = 3.0 mg/kg/day (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/day (HDT); ChE NOEL = 0.1 mg/kg/day. Carcinogenic potential negative up to 3.0 mg/kg/day (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/day (HDT); ChE NOEL = 0.1 mg/kg/day. Carcinogenic potential negative up to 3.0 mg/kg/day (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/day. 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/day 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/day for brain ChE. The NOEL for other effects, including behavioral observations, was the highest dose tested of 3.0 mg/kg/day. 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/day 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/day. 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/day, 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/day. When all assays during the first year of treatment were considered together for each group, activity of the 1.0 mg/kg/day group was nearly 50% below baseline, and the 3.0 mg/kg/day 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/day 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/day (HDT); Plasma ChE NOEL = 0.01 mg/kg/day; Plasma ChE LEL = 0.10 mg/kg; RBC AChE NOEL = 0.10 mg/kg/day; RBC AChE LEL = 1.0 mg/kg; Brain ChE NOEL = 1.0 mg/kg/day; 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., Ph.D. (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/day. 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/day (Plasma and RBC AChE inhibition at 1.0 and 5.0 mg/kg/day). Parental NOEL = 1.0 mg/kg/day (increased degree of vacuolation in zona fasciculata, especially in males; altered tinctorial properties in this tissue in females). Reproductive NOEL = 1.0 mg/kg/day (slightly reduced pup weights and slightly reduced pup survival at 5.0 mg/kg/day). There were no clinical signs specifically indicating ChE inhibition. The reproductive findings at 5 mg/kg/day 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/day in diet. ChE inhibition NOEL= 0.3 mg/kg/day. General adult toxicity NOEL = 1.0 mg/kg/day (HDT). Reproductive NOEL = 0.3 mg/kg/day (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/day (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/day (dietary). Parental toxicity NOEL = reproductive toxicity NOEL = highest dose tested = 1.2 mg/kg/day. 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/day (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/day (gavage). Maternal NOEL (excluding cholinesterase (ChE) inhibition) = 3.0 mg/kg/day (cholinergic effects). Maternal ChE inhibition NOEL = 0.1 mg/kg/day (inhibition of plasma and RBC AChE). Developmental toxicity NOEL = 15 mg/kg/day (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/day (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/day by gavage in cottonseed oil. Study demonstrates that 30 mg/kg/day 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/day. 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/day. 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/day (tremors in 3/21 dams,

transient food consumption reduction, modest but consistent body weight decrement). Developmental NOEL = 2.5 mg/kg/day (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/day 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/day 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 a.i. 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/day 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/day 3,5,6-trichloro-2-pyridinol (TCP, 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/day (minor body weight gain decrements). Developmental NOEL = 150 mg/kg/day (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/day. Maternal NOEL = 81 mg/kg/day (body weight gain decrement during treatment period). Developmental NOEL = 81 mg/kg/day [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/day. **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/day 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/day (minor maternal body weight decrement during treatment). Developmental NOEL = 25 mg/kg/day (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/day, respectively, to a historical incidence of 1/839 litters). These findings indicate a **possible adverse effect**. For perspective, 100 mg/kg/day 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/day by gavage; NOEL for maternal functional toxicity = 1 mg/kg/day [cholinesterase (ChE) effects as salivation, tremors, etc.]. ChE enzyme NOEL = 0.1 mg/kg/day (significant inhibition of maternal plasma ChE at 1 mg/kg/day). Developmental toxicity NOEL = 10 mg/kg/day (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/day (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/day.

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/day groups; NOEL (for clinical signs, FOB, motor activity) = 1 mg/kg/day. No histopathologic findings. Neuropathological NOEL = 15 mg/kg/day. **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/day (inhibition of plasma ChE in both sexes and of RBC AChE in females at 1 mg/kg/day). ChE-related clinical effects NOEL = 1 mg/kg/day (perineal staining in occasional females at 5 and 15 mg/kg/day). Motor activity reduction, at 15 mg/kg/day during the week 4 evaluation only, was confirmed statistically. NOEL for findings other than probable acute ChE effects = 15 mg/kg/day (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/day 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/day (considering a small apparent memory retention change at 10 mg/kg/day 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/day (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/day). 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/day. 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/day (brain ChE inhibition). Clinical signs of ChE inhibition were observed in 5 mg/kg/day dams. Developmental NOEL = 1 mg/kg/day (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 U.S. 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/day 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/day. No evidence of delayed distal neuropathy. 10 mg/kg/day 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/day 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/day 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

Table 1. Ten Highest Uses of Chlorpyrifos in California (lbs.), 2009-2013.

	SITE/CROP	2009	2010	2011	2012	2013	Avera
1	ALMOND	330,926	262,002	231,295	194,274	449,321	
2	CITRUS - total	175,268	241,280	267,631	177,736	211,577	
	ORANGE	119,384	*171,030*	*208,309*	*129,782*	*152,976*	*156,29*
	LEMON	32,794	*41,889*	*21,447*	*19,848*	*31,259*	*29,447*
	TANGERINE	15,814	*19,241*	*27,926*	*21,262*	*23,321*	*21,513*
	OTHER CITRUS	7,276	*9,120*	*9,949*	*6,844*	*4,021*	*7,442*
3	ALFALFA	174,301	175,866	186,063	176,343	198,126	
4	WALNUT	184,195	171,422	163,097	174,882	166,208	
5	COTTON	36,697	115,024	194,256	97,769	157,790	
6	GRAPES - total	150,568	125,168	65,754	102,434	113,916	
	WINE	*94,788*	*75,961*	*27,385*	*52,341*	*37,918*	*57,679*
	OTHER	*55,780*	*49,207*	*38,369*	*50,093*	*75,998*	*53,889*
7	CORN - total	29,629	30,599	44,929	45,535	50,478	
	ANIMAL FEED	*27,177*	*23,552*	*38,761*	*32,540*	*40,434*	*32,493*
	HUMAN CONS	*2,452*	*7,047*	*6,168*	*12,995*	*10,044*	*7,741*
8	BROCCOLI	50,072	47,391	35,509	17,419	6,985	
9	SUGARBEET - total	19,480	32,111	30,519	37,035	35,382	
	GENERAL	*19,480*	*32,111*	*30,519*	*36,910*	*35,259*	*30,856*
	TOPS (ANIMAL FEED)	*0*	*0*	*0*	*125*	*123*	*50*
10	PEACH, NECTARINE	17,731	14,257	12,135	9,335	7,405	
TOTAL USE FOR ALL SITES/CROPS		**1,247,428**	**1,284,842**	**1,300,270**	**1,106,059**	**1,467,758**	

(a) CDPR Pesticide Use Reporting (http://calpip.cdpr.ca.gov/main.cfm), accessed 11 September 2015.

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/day (placebo) for 48 days, 0.014 mg/kg/day for 27 days, 0.03 mg/kg/day for 20 days, or 0.10 mg/kg/day 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/day, 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/day 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," *Toxicology and Applied Pharmacology* **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/day 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/day 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/day, red blood cell: 0.5 mg/kg/day, brain: 0.5 mg/kg/day. 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/day, brain: 0.5 mg/kg/day. The NOEL values were reduced from 0.5 mg/kg to 0.1 mg/kg/day 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/day. 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/day and from 2 mg/kg to 0.5 mg/kg/day, 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/day) and 651 (1.0 mg/kg/day) 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/day 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 a.i. 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/day (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/day 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/day). 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% a.i.; 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 U.S. 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/day 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", **Toxicological Sciences 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/day, 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/day. 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," *Toxicological Sciences* **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/day, 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/day 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/day 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 *Toxicological Sciences* **135**(1), above, assessing the lower dose of 0.5 mg/kg/day 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/day, 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," Toxicological Sciences 122(1): 112-120 (2011). Male and female Sprague-Dawley rats were exposed to 0, 1, 2.5, or 5 mg/kg/day 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/day 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 fluorophosphonate-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/day, 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

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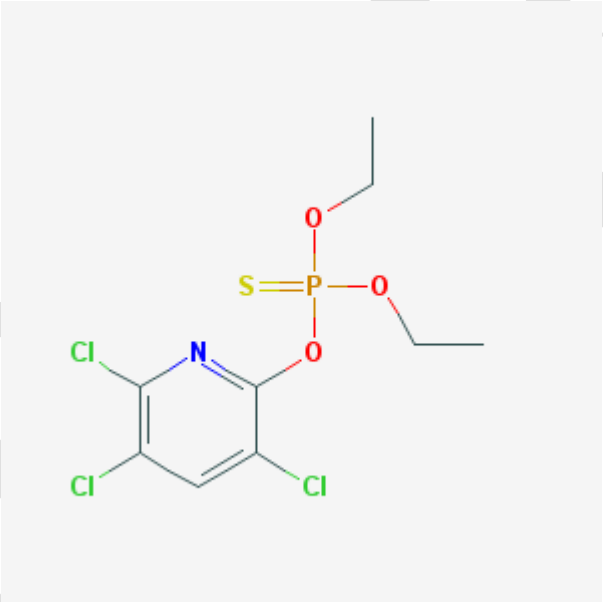
APPENDIX 2 DIETARY and DRINKING WATER EXPOSURE ASSESSMENT

CHLORPYRIFOS

Dietary Exposure Assessment:

Risk Characterization of the Acute and Steady-State Food-Only Exposures, Drinking Water Exposure Assessment, and Tolerance Assessment



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I. Introduction

The Risk Assessment Section (RAS) at the Department of Pesticide Regulation's Human Health Assessment Branch (HHAB) conducts dietary exposure assessments to evaluate the risk of human exposure to a pesticide in food and water under the mandate of Assembly Bill 2161 (Bronzan and Jones 1989). Two separate approaches are used to estimate the exposure: (1) risk is determined for the total dietary exposure based on measured residue levels on all label-approved commodities and (2) risk is determined for exposure to an individual commodity that carries pesticide residue at the tolerance level. These approaches are described in the Guidance for Dietary Exposure Assessment Dietary (DPR 2009). The RAS uses the same dietary exposure computational model, consumption databases sources for pesticide residue data, and general assumptions as the U.S. Environmental Protection Agency (U.S. EPA), but preferentially employs data from residue-monitoring programs with a California component that are expected to be more representative of residue levels within the State.

In 2014, the U.S. EPA conducted highly refined probabilistic acute and steady-state (21-day) dietary (food-only) exposure assessments of chlorpyrifos using two models: (1) the Dietary Exposure Evaluation Model-Food Commodity Ingredient Database (DEEM-FCID™, version 2.036 (acute exposure), and (2) Calendex-FCID (21-day, steady state exposure). Both models incorporate food consumption data from the National Health and Nutrition Examination Survey (NHANES) for 2003-2008 (USEPA 2014a, 2015). U.S. EPA's 2014 dietary exposure assessment follows a preliminary exposure assessment that was released in 2011 (USEPA 2011a). It did not calculate risks from total dietary exposure, but provided food-only exposure values for use in the aggregate risk assessment. The U.S. EPA addressed the exposure from drinking water in separate drinking water assessments (DWA) (USEPA 2011b, 2014b, c).

RAS reviewed the U.S. EPA dietary and drinking water exposure assessments. Because no new uses for chlorpyrifos have been introduced since those assessments were published in December 2014, RAS determined that it is not necessary to conduct an independent dietary exposure assessment at this time. U.S. EPA's DWA was incomplete and did not contain modeling data specific for California. Therefore, RAS utilized the 2014 U.S. EPA's food-only exposure estimates to evaluate the risk from chlorpyrifos exposure from food and conducted its own drinking water exposure assessment employing DPR residue data from surface water in California and PDP monitoring data for drinking water in California.

II. Food-Only Exposure Assessment

II.A. Description of Dietary Exposure Assessment Models

3) DEEM-FCID

DEEM-FCID is a computer program for estimating exposure and/or risk to human health from pesticides in food (USEPA 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 U.S. 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.

4) 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 U.S. 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 chlorpyrifos (USEPA 2014b).

II.B. Residue Data and Refinements

Chlorpyrifos 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 (2009-2013), the top ten agricultural uses in the state were almond, citrus, alfalfa, walnut, cotton, grapes, corn, broccoli, sugar beet, and peach/nectarine. Average annual use for all sites, including all agricultural and non-agricultural uses, was 1.3 million lbs/year (Table 1).

U.S. EPA tolerances for residues of chlorpyrifos 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; Table 2). Two of the tolerances, for grape and asparagus, are regional. Chlorpyrifos-oxon residues are not included in the tolerances established for chlorpyrifos residues because it is generally not found in food.

U.S. 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 chlorpyrifos 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. Chlorpyrifos in not registered for use on potatoes in the U.S. (USEPA 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 it was appropriate. The following commodities had no detects of chlorpyrifos 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, U.S. EPA's analysis used either 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 U.S. 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.

Exposure estimates were compared to population-adjusted doses (PADs) in the U.S. EPA evaluation. PADs were based on points of departure that were estimated from physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) modeling of RBC cholinesterase inhibition in humans.

Table 1. Ten Highest Uses of Chlorpyrifos in California (lbs.), 2009-2013.

	SITE/CROP	2009	2010	2011	2012	2013	Average, 2009-2013
1	ALMOND	330,926	262,002	231,295	194,274	449,321	293,564
2	CITRUS - total	175,268	241,280	267,631	177,736	211,577	214,698
	ORANGE	119,384	*171,030*	*208,309*	*129,782*	*152,976*	*156,296*
	LEMON	32,794	*41,889*	*21,447*	*19,848*	*31,259*	*29,447*
	TANGERINE	15,814	*19,241*	*27,926*	*21,262*	*23,321*	*21,513*
	OTHER CITRUS	7,276	*9,120*	*9,949*	*6,844*	*4,021*	*7,442*
3	ALFALFA	174,301	175,866	186,063	176,343	198,126	182,140
4	WALNUT	184,195	171,422	163,097	174,882	166,208	171,961
5	COTTON	36,697	115,024	194,256	97,769	157,790	120,307
6	GRAPES - total	150,568	125,168	65,754	102,434	113,916	111,568
	WINE	*94,788*	*75,961*	*27,385*	*52,341*	*37,918*	*57,679*
	OTHER	*55,780*	*49,207*	*38,369*	*50,093*	*75,998*	*53,889*
7	CORN - total	29,629	30,599	44,929	45,535	50,478	40,234
	ANIMAL FEED	*27,177*	*23,552*	*38,761*	*32,540*	*40,434*	*32,493*
	HUMAN CONS	*2,452*	*7,047*	*6,168*	*12,995*	*10,044*	*7,741*
8	BROCCOLI	50,072	47,391	35,509	17,419	6,985	31,475
9	SUGARBEET - total	19,480	32,111	30,519	37,035	35,382	30,905
	GENERAL	*19,480*	*32,111*	*30,519*	*36,910*	*35,259*	*30,856*
	TOPS (ANIMAL FEED)	*0*	*0*	*0*	*125*	*123*	*50*
10	PEACH, NECTARINE	17,731	14,257	12,135	9,335	7,405	12,173
TOTAL USE FOR ALL SITES/CROPS		**1,247,428**	**1,284,842**	**1,300,270**	**1,106,059**	**1,467,758**	**1,281,271**

(a) CDPR Pesticide Use Reporting (http://calpip.cdpr.ca.gov/main.cfm), accessed 11 September 2015.

Table 2. U.S. EPA tolerances for Chlorpyrifos residues as of December 2015.

	Commodity	Tolerance (ppm)
1	Alfalfa, forage	3.0
2	Alfalfa, hay	13
3	Almond	0.2
4	Almond, hulls	12
5	Apple	0.01
6	Apple, wet pomace	0.02
7	Banana	0.1
8	Beet, sugar, dried pulp	5.0
9	Beet, sugar, molasses	15
10	Beet, sugar, roots	1.0
11	Beet, sugar, tops	8.0
12	Cattle, fat	0.3
13	Cattle, meat	0.05
14	Cattle, meat byproducts	0.05
15	Cherry, sweet	1.0
16	Cherry, tart	1.0
17	Citrus, dried pulp	5.0
18	Citrus, oil	20
19	Corn, field, forage	8.0

Table 2. U.S. EPA tolerances for Chlorpyrifos residues as of December 2015.		
	Commodity	**Tolerance (ppm)**
20	Corn, field, grain	0.05
21	Corn, field, refined oil	0.25
22	Corn, field, stover	8.0
23	Corn, sweet, forage	8.0
24	Corn, sweet, kernel plus cob with husk removed	0.05
25	Corn, sweet, stover	8.0
26	Cotton, undelinted seed	0.2
27	Cranberry	1.0
28	Cucumber	0.05
29	Egg	0.01
30	Fig	0.01
31	Fruit, citrus, group 10	1.0
32	Goat, fat	0.2
33	Goat, meat	0.05
34	Goat, meat byproducts	0.05
35	Hazelnut	0.2
36	Hog, fat	0.2
37	Hog, meat	0.05
38	Hog, meat byproducts	0.05
39	Horse, fat	0.25

Table 2. U.S. EPA tolerances for Chlorpyrifos residues as of December 2015.

	Commodity	Tolerance (ppm)
40	Horse, meat	0.25
41	Horse, meat byproducts	0.25
42	Kiwifruit	2.0
43	Milk, fat (Reflecting 0.01 ppm in whole milk)	0.25
44	Nectarine	0.05
45	Onion, bulb	0.5
46	Peach	0.05
47	Peanut	0.2
48	Peanut, refined oil	0.2
49	Pear	0.05
50	Pecan	0.2
51	Pepper	1.0
52	Peppermint, tops	0.8
53	Peppermint, oil	8.0
54	Plum, prune, fresh	0.05
55	Poultry, fat	0.1
56	Poultry, meat	0.1
57	Poultry, meat byproducts	0.1
58	Pumpkin	0.05
59	Radish	2.0

Table 2. U.S. EPA tolerances for Chlorpyrifos residues as of December 2015.

	Commodity	Tolerance (ppm)
60	Rutabaga	0.5
61	Sheep, fat	0.2
62	Sheep, meat	0.05
63	Sheep, meat byproducts	0.05
64	Spearmint, tops	0.8
65	Spearmint, oil	8.0
66	Sorghum, grain, forage	0.5
67	Sorghum, grain, grain	0.5
68	Sorghum, grain, stover	2.0
69	Soybean, seed	0.3
70	Strawberry	0.2
71	Sunflower, seed	0.1
72	Sweet potato, roots	0.05
73	Turnip, roots	1.0
74	Turnip, tops	0.3
75	Vegetable, brassica, leafy, group 5	1.0
76	Vegetable, legume, group 6. except soybean	0.05
77	Walnut	0.2
78	Wheat, forage	3.0
79	Wheat, grain	0.5

Table 2. U.S. EPA tolerances for Chlorpyrifos residues as of December 2015.		
	Commodity	**Tolerance (ppm)**
80	Wheat, straw	6.0
	Tolerances with regional registrations	
81	Asparagus	5.0
82	Grape	0.01

(CFR 40 §180.342, current as of December 11, 2015; http://www.ecfr.gov/cgi-bin/text-idx?SID=c7f0c0c16a825c8cd9d3bdff4c7b51c3&mc=true&node=se40.24.180_1342&rgn=div8).

II.C. Results of the U.S. EPA Food-Only Exposure Assessment

Exposure estimates from the 2014 U.S. EPA assessment are shown in Table 3. Children 1-2 years old were identified as the highest exposed population subgroup: at the 99.9th percentile, exposure was 0.000423 mg/kg.

Although a commodity contribution analysis was not included in either the 2011 or 2014 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 3. Acute dietary (food only) exposure analysis (all subpopulations) for chlorpyrifos (a).

ACUTE EXPOSURE, FOOD ONLY			
Population Subgroup (b)	**Exposure (mg/kg/day)**		
	95th Percentile	**99th Percentile**	**99.9th Percentile**
General U.S. Population	0.000031	0.000064	0.000197
All Infants (< 1 year old)	0.000050	0.000088	0.000273
Children 1-2 years old	0.000082	0.000143	0.000423
Children 3-5 years old	0.000062	0.000107	0.000319
Children 6-12 years old	0.000040	0.000072	0.000189
Youth 13-19 years old	0.000024	0.000042	0.000126
Adults 20-49 years old	0.000021	0.000042	0.000167
Adults 50-99 years old	0.000022	0.000044	0.000186
Females 13-49 years old	0.000021	0.000041	0.000150

(a) Data are from U.S. EPA's 2014 dietary exposure assessment to support registration review (USEPA 2014b).
(b) Sentinel populations are shaded.

II.D. RAS Risk Characterization of the Food-Only Exposure

RAS evaluated the risk from exposure to chlorpyrifos residues in food by estimating the margin of exposure (MOE), a quotient of the critical No Observable Effects Level (NOEL) and the human exposure level. The critical NOELs (toxicological point of departures, PoDs) for chlorpyrifos were PBPK-PD-estimated human equivalent doses. Methodology for deriving the PoDs is described in detail in the RCD (III. HAZARD IDENTIFICATION, see Table 18). The PoDs for the sentinel populations evaluated for dietary risk to chlorpyrifos are shown in Tables 4 and 5 for acute and steady-state exposures, respectively. A calculated MOE of 100 was considered prudent for protection against chlorpyrifos toxicity. The target of 100 includes an uncertainty factor of 1 for interspecies sensitivity, 10 for intraspecies variability and 10 for potential neurodevelopmental effects (see RCD under V. RISK CHARACTERIZATION- MOE and Risk Calculations).

The acute dietary MOEs were estimated using acute PoDs and the probabilistic exposures in Table 4. At the 99.9th percentile, the MOEs were greater than the target of 100 for all four population subgroups.

The steady-state dietary MOEs were estimated using the steady state PoDs (ssPoDs) and the probabilistic exposures in Table 5. At the 99.9th percentile, the MOEs were greater than the target of 100 for all four population subgroups.

Table 4. Acute dietary (food only) exposure and Margins of Exposure (MOE, sentinel populations) for chlorpyrifos.

ACUTE EXPOSURE, FOOD ONLY (a)							
Population Subgroup	aPoD (mg/kg) (c)	95th Percentile		99th Percentile		99.9th Percentile	
		Exposure (mg/kg/day)	MOE (b)	Exposure (mg/kg/day)	MOE (b)	Exposure (mg/kg/day)	MOE (b)
All Infants (< 1 year old)	0.600	0.000050	12,000	0.000088	6,818	0.000273	2,198
Children 1-2 years old	0.581	0.000082	7,085	0.000143	4,063	0.000423	1,374
Children 6-12 years old	0.530	0.000040	13,250	0.000072	7,361	0.000189	2,804
Females 13-49 years old	0.469	0.000021	22,333	0.000041	11,439	0.000150	3,127

(a) Exposures are from the U.S. EPA's 2014 dietary exposure assessment to support registration review (USEPA 2014b).
(b) Target MOE is 100 for every population.
(c) Point of Departures are PBPK-PD-estimated human equivalent doses .

Table 5. Steady state (21-day) dietary (food only) exposure and Margins of Exposure (MOE, sentinel populations) for chlorpyrifos.

STEADY STATE EXPOSURE, FOOD ONLY							
Population Subgroup	ssPoD (mg/kg)	70th Percentile		95th Percentile		99.9th Percentile	
		Max.Exposure (mg/kg)	MOE (a)	Max.Exposure (mg/kg)	MOE (a)	Max.Exposure (mg/kg)	MOE (a)
All Infants (< 1 year old)	0.103	0.000020	5,150	0.000045	2,289	0.000186	554
Children 1-2 years old	0.099	0.000038	2,605	0.000072	1,375	0.000242	409
Children 6-12 years old	0.090	0.000019	4,737	0.000039	2,308	0.000128	703
Females 13-49 years old	0.078	0.000009	8,667	0.000018	4,333	0.000075	1,040

(a) Target MOE is 100 for every population.
(b) Data are from U.S. EPA's 2014 dietary exposure assessment to support registration review (USEPA 2014b).
(c) Point of Departures are PBPK-PD-estimated human equivalent doses.

III. Drinking Water Exposure Assessment

III.A. Summary of U.S. EPA Drinking Water Assessments

U.S. EPA conducted a preliminary drinking water assessment (DWA) in 2011 and updated it with additional analyses in 2014 (USEPA 2011a, 2014c). Chlorpyrifos is rapidly oxidized to the oxon during the chlorination process of drinking-water treatment. Since more than 75% of community water systems in the U.S. use chlorination to disinfect drinking water, the DWA assessment assumed that chlorpyrifos is converted 100% to chlorpyrifos oxon during water treatment processes. A drinking water level of concern (DWLOC) of 3.9 ppb was calculated for exposure to chlorpyrifos 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 groundwater 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 of them (except a registrant study of Orestimba Creek in Stanislaus County) were capable of detecting peak or 21-day average concentrations of chlorpyrifos or chlorpyrifos-oxon because the frequency of monitoring did not coincide with either the exposure period of interest or the timing of chlorpyrifos applications.

- **Drinking water derived from groundwater (i.e., wells) is predicted[5] to have acceptable levels** of chlorpyrifos and chlorpyrifos-oxon. Even for a use scenario with 5 applications per year totaling 14.5 lbs chlorpyrifos per acre, 21-day average concentration of chlorpyrifos-oxon in drinking water derived from groundwater is not expected to be greater than 0.15 µg / L (USEPA 2014c, Table 1 and page 13). That is less than 4% of the Drinking Water Level of Concern (DWLOC) of 3.9 µg / L for chlorpyrifos-oxon[6].

- **Drinking water derived from surface water is predicted[7] to pose an exposure concern (Table 1, and Figure 3).** "Several chlorpyrifos 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" (USEPA 2014c) Uses that may exceed the DWLOC include scenarios for certain California cropping systems, e.g. wheat, rangeland, cole crops, and wine grapes (Figure 3).

- **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 chlorpyrifos 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[8].

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[5] **For drinking water derived from groundwater, 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 Groundwater (SCI-GROW) version 2.3, and Pesticide Root Zone Model for GroundWater (PRZM-GM). A previous evaluation by U.S. EPA showed that, "In a few cases PRZM-GM underestimated pesticide concentration observed in groundwater", 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.

[6] **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 U.S. EPA's 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 USEPA 2014c, the value 217 ppb corresponds to infants, the most susceptible population; see U.S. EPA 2014 chlorpyrifos risk assessment (USEPA 2014b) Table 4.8.4. The 50x "safety factors" used by Bohaty (USEPA 2014c) comprise 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: U.S. EPA 2014 chlorpyrifos risk assessment (USEPA 2014b), p. 8.

[7] **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 GroundWater 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." Quote is from USEPA 2014c p. 14.

[8] **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

- **Routine treatment of drinking water is <u>not</u> expected to mitigate the risk.** The following quotes are from USEPA 2014c. "In general, drinking water treatment processes, with the exception of activated carbon, have been shown to have little impact on removal of pesticide residues." "It is possible that some drinking water treatment procedures, such as granular activated carbon filtration and water softening (increased rate of chlorpyrifos-oxon hydrolysis at pH > 9) could reduce the amount of chlorpyrifos-oxon in finished drinking water; however, these treatment methods are not typical practices across the country." "All the chlorpyrifos that enters a drinking water treatment facility is assumed to be converted to chlorpyrifos-oxon during treatment [chlorination]. Although chlorpyrifos-oxon has a hydrolysis half-life of 5 days, the drinking water treatment simulation half-life for chlorpyrifos-oxon is approximately 12 days. Therefore, once chlorpyrifos-oxon forms during treatment, little transformation is expected to occur before consumption (during drinking water distribution)."

III.B. RAS Evaluation of the Exposure to Chlorpyrifos in Drinking Water in California

In the absence of modeling data specific for California, RAS utilized residue data from PDP's drinking water study (PDP 2015) and from the testing of surface and ground water in California to evaluate the potential exposure to chlorpyrifos through drinking water.

III.B.1. 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 groundwater. A total of 1,835 samples were analyzed for chlorpyrifos and/or chlorpyrifos oxon and no residues were detected. LODs ranged from 3 to 30 ppt for chlorpyrifos and 12 to 510 ppt for chlorpyrifos oxon (Table 6). The average LOD for chlorpyrifos oxon in finished (treated) water samples (n = 706) was 38.2 ppt.

Exposure to chlorpyrifos oxon in drinking water was estimated by assuming that each of the 706 samples of finished (treated) water contained chlorpyrifos oxon at concentrations equivalent to the LOD for chlorpyrifos 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 7).

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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" (USEPA 2014c, pp. 7-8).

Table 6. PDP monitoring data for chlorpyrifos (CPF) and chlorpyrifos oxon (CPO) in groundwater, untreated drinking water, finished (treated) drinking water, and bottled water in California, 2001-2013 (PDP 2015).

YEAR	CHEMICAL	SAMPLE TYPE	SAMPLES	DETECTS	LOD (PPT)
2001	CPF	Finished	134	0	11
	CPO	Finished	134	0	20
2002	CPF	Finished	267	0	6-9
	CPO	Finished	265	0	12
2003	CPF	Finished	272	0	9
	CPO	Finished	272	0	12
2004	no data ==>				
2005	CPF	Bottled	93	0	30
	CPF	Finished	26	0	11
	CPF	Untreated	28	0	11
	CPO	Finished	26	0	510
	CPO	Untreated	28	0	510
2006	CPF	Bottled	88	0	30
	CPF	Finished	9	0	11
	CPF	Untreated	9	0	11
	CPO	Finished	9	0	510
	CPO	Untreated	9	0	510
2007	CPF	Groundwater	4	0	30
2008	CPF	Groundwater	2	0	30
2009	CPF	Groundwater	13	0	30
2010	CPF	Groundwater	27	0	30
2012	CPF	Untreated	26	0	3-30
	CPF	Finished	26	0	3-30
	CPO	Untreated	26	0	12-21
	CPO	Finished	26	0	12-21
2013	CPF	Groundwater	8	0	30
	CPO	Groundwater	8	0	12

LOD = limit of detection.

Table 7. DEEM-FCID (v. 3.18) Acute Exposure Estimates for Chlorpyrifos Oxon in Drinking Water Based on 2001-2013 PDP Residue Data for Chlorpyrifos Oxon in Treated (Finished) Water (a)			
Probabilistic Estimate With All Non-Detects at the LOD (b)			
Population Subgroup	**Exposure (mg/kg/day)**		
	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". Samples with non-detectible residues were assumed to contain chlorpyrifos oxon at the LOD.			
(b) 706 samples, no detections. LODs ranged 12-510 ppt (mean = 38.2 ppt).			

III.B.2. Analysis of Drinking Water Exposure Using DPR Surface Water Residue Data

The Environmental Monitoring Branch (EMON) at DPR collects residue data from sampling of surface water within California by a number of government agencies including USGS, State Water Resources Control Board, and CALFED Bay-Delta Program, as well as sampling by DPR. 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 7,154 samples of California surface water were analyzed for chlorpyrifos 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 chlorpyrifos oxon and there were no detected residues (average detection limit ranged from 0.05 to 0.08 ppb) (Table 8) (DPR 2015a).

Exposure to chlorpyrifos oxon in drinking water was estimated by conducting a probabilistic analysis using either the detected chlorpyrifos 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 7,048 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 9). These exposures were up to 4-fold higher than the exposures estimated based on the PDP monitoring data.

Table 8: Summary of Surface Water Monitoring for Chlorpyrifos in California, 2005 - 2014.

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

CPF = chlorpyrifos, CPO = chlorpyrifos oxon.

Table 9. DEEM-FCID (v. 3.18) Acute Exposure Estimates for Chlorpyrifos Oxon in Drinking Water Based on 2005-2014 Surface Water Residue Data (a)			
Probabilistic Estimate With All Non-Detects at the Detection Limit (b)			
Population Subgroup	**Exposure (mg/kg/day) (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). Det. limit ranged 0.001 - 4 ppb, mean = 0.045 ppb).			
(c) Chlorpyrifos exposure values were converted to chlorpyrifos oxon by applying a molecular weight correction factor (0.9541).			

III.B.3. Analysis of Drinking Water Exposure Using DPR Ground Water Residue Data

The EMON branch at DPR collects residue data from sampling of groundwater within California by a number of government agencies including U.S. 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 2,055 samples were analyzed for chlorpyrifos 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 1,903 samples were analyzed for chlorpyrifos oxon and there were no detected residues (average detection limit ranged from 0.05 to 0.06 ppb) (Table 10) (DPR 2015b).

Exposure to chlorpyrifos oxon in drinking water was estimated by conducting a probabilistic analysis using either the detected chlorpyrifos residue in groundwater 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 2,055 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 11).

Table 10: Summary of Groundwater Monitoring for Chlorpyrifos 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
	CPO	151	0	0.0%	n/a	0.0560
2005	CPF	388	0	0.0%	n/a	0.0050
	CPO	388	0	0.0%	n/a	0.0560
2006	CPF	478	2	0.0%	0.006 - 0.008	0.0071
	CPO	477	0	0.0%	n/a	0.0560
2007	CPF	354	0	0.0%	n/a	0.0107
	CPO	352	0	0.0%	n/a	0.0560
2008	CPF	437	0	0.0%	n/a	0.0921
	CPO	395	0	0.0%	n/a	0.0553
2009	CPF	94	0	0.0%	n/a	0.0837
	CPO	78	0	0.0%	n/a	0.0500
2010	CPF	65	0	0.0%	n/a	0.0862
	CPO	60	0	0.0%	n/a	0.0500
2011	CPF	46	0	0.0%	n/a	0.9393
	CPO	2	0	0.0%	n/a	0.0600
2012	CPF	22	0	0.0%	n/a	1.0000
	CPO	0	n/a	n/a	n/a	n/a
2013	CPF	25	0	0.0%	n/a	1.0000
	CPO	0	n/a	n/a	n/a	n/a

CPF = chlorpyrifos, CPO = chlorpyrifos oxon.

Table 11. DEEM-FCID (v. 3.18) Acute Exposure Estimates for Chlorpyrifos Oxon in Drinking Water Based on 2004-2013 Groundwater Residue Data (a)			
Probabilistic Estimate With All Non-Detects at the Detection Limit (b)			
Population Subgroup	**Exposure (mg/kg/day) (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, two detections (0.006, 0.008 ppb). Det. limit for non-detects ranged 0.004 - 1 ppb (mean = 0.072 ppb).			
(c) Chlorpyrifos exposure values were converted to chlorpyrifos oxon by applying a molecular weight correction factor (0.9541).			

III.B.4. Risk Characterization of the Drinking Water Exposure

Table 12 shows acute MOEs for exposure to chlorpyrifos 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 chlorpyrifos 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 12. However, lack of residue data precludes a steady-state drinking water assessment at this time.

Table 12. Summary of acute dietary (water only) 99.9th percentile exposures and Margins of Exposure (MOE, sentinel populations) for chlorpyrifos oxon.

ACUTE EXPOSURE, WATER ONLY (a)							
Population Subgroup	aPoD (mg/kg) (c)	PDP		Surface Water		Groundwater	
		Exposure (mg/kg/day)	MOE (b)	Exposure (mg/kg/day)	MOE (b)	Exposure (mg/kg/day)	MOE (b)
All Infants (< 1 year old)	0.170	0.000108	1,571	0.000419	405	0.000222	764
Children 1-2 years old	0.159	0.000057	2,791	0.000177	899	0.000115	1,384
Children 6-12 years old	0.143	0.000036	3,970	0.000110	1,299	0.000075	1,905
Females 13-49 years old	0.129	0.000036	3,588	0.000119	1,085	0.000073	1,769

(a) 99.9th percentile of exposure from probabilistic analysis using residue data from PDP or CDPR surface and groundwater databases.
(b) Target MOE is 100 for every population.
(c) Point of Departures are PBPK-PD-estimated human equivalent doses .

IV. Tolerance Assessment

A tolerance is the legal maximum residue concentration of a pesticide that is allowed on a raw agricultural commodity or processed food. Tolerances are established at levels necessary for the maximum application rate and frequency, and not expected to produce deleterious health effects in humans from chronic dietary exposure (U.S. EPA, 1991). U.S. EPA is responsible for setting tolerances for pesticide residues in raw agricultural commodities (Section 408 of FFDCA) and processed commodities (Section 409 of the Federal Food, Drug, and Cosmetic Act (FFDCA). The data requirements for tolerances include: (1) residue chemistry, (2) environmental fate, (3) toxicology, (4) product performance such as efficacy, and (5) product chemistry (Code of Federal Regulations, 1996). Field studies must reflect the proposed use with respect to the rate and mode of application, number and timing of applications and formulations proposed (U.S. EPA, 1982.)

In 1996, the Food Quality Protection Act (FQPA) amended the overall regulation of pesticide residues under FIFRA and FFDCA (U.S. EPA, 1997). One major change was the removal of the Delaney Clause that prohibited residues of cancer-causing pesticides in processed foods. Tolerances must be health-based and the same standards are used to establish tolerances for both the raw agricultural commodities and their processed forms. FQPA required an explicit finding that tolerances are safe for children. U.S. EPA was required to use an extra 10-fold safety factor to take into account potential pre- and postnatal developmental toxicity unless they determined, based on reliable data, that a different margin would be safe. In addition, evaluations of the tolerance must take into account: (1) aggregate exposure from all non-occupational sources, (2) effects from cumulative exposure to the pesticide and other substances with common mechanisms of toxicity, (3) effects of *in utero* exposure; and (4) potential for endocrine disrupting effects. Under FQPA, U.S. EPA was also required to reassess all existing tolerances and exemptions from tolerances for both active and inert ingredients by 2006 (U.S. EPA, 1997). Previously, they reassessed tolerances as part of its reregistration and Special Review processes. In the evaluation of tolerances, U.S. EPA uses a tiered approach and the assessment includes all label-use commodities. Tolerances for chlorpyrifos were last reassessed in 2006 in conjunction with the cumulative risk assessment for organophosphates.

In California, U.S. EPA established tolerances are evaluated under the mandate of Assembly Bill 2161 (Bronzan and Jones, 1989). The Act requires DPR to conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides. When the risk is considered deleterious to human health, DPR can promulgate regulations to mitigate the exposure.

The tolerance assessment is conducted for a single individual label-approved commodity (DPR, 2009). The commodities are selected with potential for high exposures based on commodity contribution analyses. Exposures are presented at the 95th percentile exposure to the individual commodity with the residue level set at the tolerance.

Acute Dietary Exposure. For chlorpyrifos, tolerances for the following commodities were evaluated: apple, banana, bell pepper, broccoli, cabbage, sweet corn, grapefruit, onion (bulb), orange, and strawberry (Table 13). These commodities were selected because of high consumption rates or high contribution to exposure in U.S. EPA's 2011 preliminary dietary exposure assessment. MOEs were evaluated for the four sentinel populations.

The evaluated commodities with the least dietary exposure at tolerance were apple, bell pepper, sweet corn, onion, and strawberry. These exposures resulted in MOEs higher than the target of 100 for all four populations. In contrast, MOEs were lower than the target of 100 for one or more population subgroups exposed to a tolerance level of chlorpyrifos on banana, broccoli, cabbage, grapefruit, and orange (Table 13).

Chronic Dietary Exposure. A chronic exposure assessment using residues equal to the established tolerances for individual commodities or combinations of commodities was not conducted because it is highly improbable that an individual would habitually consume single or multiple commodities with pesticide residues at the tolerance levels.

TABLE 13: Acute Margins of Exposure at 95th Percentile Consumption Rate for Single Commodities With Tolerance Level Residues (a)

COMMODITY (b)	TOLERANCE		All Infants	Children 1-2 y	Children 6-12 y	Females 13-49 y
		aPoD (mg/kg) =	0.600	0.581	0.530	0.469
Apple	0.01	*consumption*	0.037647	0.043133	0.013620	0.005565
		MOE	1,594	1,347	3,891	8,428
Banana	0.1	*consumption*	0.015830	0.011633	0.004687	0.002329
		MOE	379	499	1,131	2,014
Pepper, bell	1.0	*consumption*	0.000373	0.001268	0.000936	0.000759
		MOE	1,609	458	566	618
Broccoli	1.0	*consumption*	0.007739	0.009570	0.005523	0.002790
		MOE	78	61	96	168
Cabbage	1.0	*consumption*	0.003146	0.006809	0.006474	0.002469
		MOE	191	85	82	190
Corn, sweet	0.05	*consumption*	0.005068	0.007179	0.005702	0.002446
		MOE	2,368	1,619	1,859	3,835
Grapefruit	1.0	*consumption*	0.004688	0.003571	0.000552	0.003726
		MOE	128	163	960	126
Onion, bulb	0.5	*consumption*	0.001012	0.001352	0.000889	0.000658
		MOE	1,186	859	1,192	1,426
Orange	1.0	*consumption*	0.013526	0.030216	0.013319	0.009278
		MOE	44	19	40	51
Strawberry	0.2	*consumption*	0.002774	0.005247	0.002513	0.001585
		MOE	1,081	554	1,055	1,479

(a) MOE = aPoD/(tolerance x consumption). Target MOE = 100 for every subpopulation. Shaded cells indicate MOEs less than target.

(b) Commodities were selected from 21CFR101.44 (2012), "Most frequently consumed raw fruits, vegetables, and fish in the United States". 95th percentile consumption rates (kg/kg) are from DEEM-FCID, v. 3.1 (NHANES 2003-2008) and include all food forms (fresh, dried, juice, etc.).

V. Risk Appraisal

V.A. 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, the DPR's residue monitoring program detected illegal chlorpyrifos residues on the commodities shown in Table 14. A high proportion of samples of cactus (leaves or fruit), litchi, and longan contained illegal chlorpyrifos residues. 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. It should be noted that the goal of DPR's program is regulatory compliance, so samples are prepared according to the tolerance definition (usually "in or on"), while the PDP program is designed for dietary risk assessment so standard consumer practices such as rinsing are followed and only the edible portion of samples is analyzed for pesticide residues. Therefore, DPR's monitoring may detect illegal residues more frequently or at higher concentrations than those detected by PDP.

RAS does not evaluate illegal residues on agricultural commodities in its dietary exposure assessments. However the high frequency of these detections for chlorpyrifos suggests there could be additional exposures not considered in the dietary assessment.

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 U.S. EPA's exposure values, which were estimated on a per capita (all individuals surveyed) basis. RAS selects per user-day (consumers only or the population that is exposed) basis for the acute exposure rather than the entire population (per capita) (DPR 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.

Table 14: Commodities sampled by DPR's pesticide residue monitoring program that had illegal chlorpyrifos residues, 2012-2014 (a)

COMMODITY NAME	NUMBER OF SAMPLES TESTED	NUMBER OF SAMPLES WITH ILLEGAL RESIDUES	% WITH ILLEGAL RESIDUES	--- SAMPLES WITH ILLEGAL RESIDUES ---		
				MINIMUM CONC. (ppm)	MAXIMUM CONC. (ppm)	AVERAGE CONC. (ppm)
BEANS, ASPARAGUS	67	1	1.49%	0.66	0.66	0.66
CACTUS, LEAVES OR FRUIT	164	16	9.76%	0.022	0.29	0.093
CARAMBOLA	14	1	7.14%	0.05	0.05	0.05
CELERY	83	1	1.20%	0.02	0.02	0.02
CHINESE AMARANTH	4	1	25.00%	0.03	0.03	0.03
CILANTRO	126	3	2.38%	0.02	0.04	0.033
DILL	5	2	40.00%	0.026	0.075	0.05
LETTUCE, LEAF	121	1	0.83%	0.02	0.02	0.02
LITCHI	19	6	31.58%	0.044	0.21	0.11
LONGAN	21	6	28.57%	0.039	0.2	0.1
PEACH	316	2	0.63%	0.1	0.13	0.12
PEAR	242	3	1.24%	0.059	0.091	0.078
PEPPERS (CHILI TYPE)	211	1	0.47%	1.68	1.68	1.68
SPINACH	409	3	0.73%	0.02	0.09	0.063
SUBTROPICAL AND TROPICAL FRUIT (UNSPEC)	15	1	6.67%	0.058	0.058	0.058
SWISS CHARD	31	2	6.45%	0.22	1.29	0.755
TARO	31	2	6.45%	0.032	0.1	0.066
TOMATILLO	301	11	3.65%	0.02	0.15	0.058

(a) An illegal residue is one that either exceeds the U.S. tolerance or is detected on a commodity that has no tolerance for the subject pesticide.

V.B. Issues Related to Drinking Water Exposure

U.S. 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.

RAS 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 groundwater 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 are designed to 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 biased toward capturing 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 may not be used as sources for drinking water. Consequently, a drinking water exposure based on these residues would likely represent the "high-end" of the potential exposure. Regardless of the residue database, all acute drinking water MOEs at the 99.9th 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.

VI. References

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APPENDIX 3. ESTIMATION OF CHLORPYRIFOS HORIZONTAL DEPOSITION AND AIR CONCENTRATIONS

Barry TA. 2015. Estimation of Chlorpyrifos Horizontal Deposition and Air Concentrations for California Use Scenarios. Department of Pesticide Regulation. California Environmental Protection Agency. Sacramento, CA 95812.

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SUBJECT: Estimation of Chlorpyrifos Horizontal Deposition and Air Concentrations for California Use Scenarios

Background

This memorandum describes procedures used to estimate chlorpyrifos off-site horizontal deposition and air concentrations associated with California use scenarios. These estimates are suitable for use in conducting chlorpyrifos human exposure assessments and in developing exposure mitigation measures for the use of chlorpyrifos. 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). United States Environmental Protection Agency (USEPA) Office of Pesticide Programs (OPP) uses AgDRIFT for all agricultural deposition analysis and uses AGDISP for mosquito adulticide application scenarios (U.S.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 air blast 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 (http://www.epa.gov/scipoly/sap/meetings/1997/december/spraydrift.htm). 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. The latest "public" version of AgDRIFT 2.1.1 executable file dated 01/2012 was sent to staff following a request for the latest version of the model through the www.agdrift.com webmaster. However, it was discovered that this public version of AgDRIFT 2.1.1 does not have several capabilities that the older version includes. Specifically, for orchard airblast this public version of the model does not allow access to the extended settings for specific orchard types (e.g. dormant apples) and for ground boom the 90th percentile estimates are not available. AgDRIFT 2.0.05 is an older version of the model but produces deposition results identical to the public version accessible scenarios for all application methods (aerial, ground boom, and orchard airblast). In addition, the 90th percentile ground boom results obtained from AgDRIFT 2.0.05 were identical to the deposition results shown in the recent USEPA guidance on spray drift (White et al., 2013) that USEPA produced using the regulatory version of AgDRIFT 2.1.1. After the analyses in this memorandum were completed, staff was able to obtain a copy of the AgDRIFT 2.1.1 regulatory version, executable filed dated 12/2011. As expected, results from this version of the model were identical to AgDrift 2.0.05 and the public version of AgDRIFT 2.1.1.

The AGDISP 8.28 model was used for aerial application deposition 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 has 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). Staff originally had access only to the public version of the most recent release, AgDRIFT 2.1.1. This most recent public version of AgDRIFT does not include a time step improvement recently incorporated into AGDISP 8.28 (M. Teske, pers. comm., 2014). The lack of that time step improvement in the public version of AgDRIFT 2.1.1 will result 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 2.28.

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 USEPA default (U.S.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 ac. A preliminary deposition limit of 0.35% of the application rate was used for initial drift model scenairo scoping (S. Beauvais, pers. comm., 2014).

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 STDF 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 deposition. 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 below. 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). Based upon the greatest distance to the preliminary deposition level of 0.35% of application rate, 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 1.2xrotor 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.

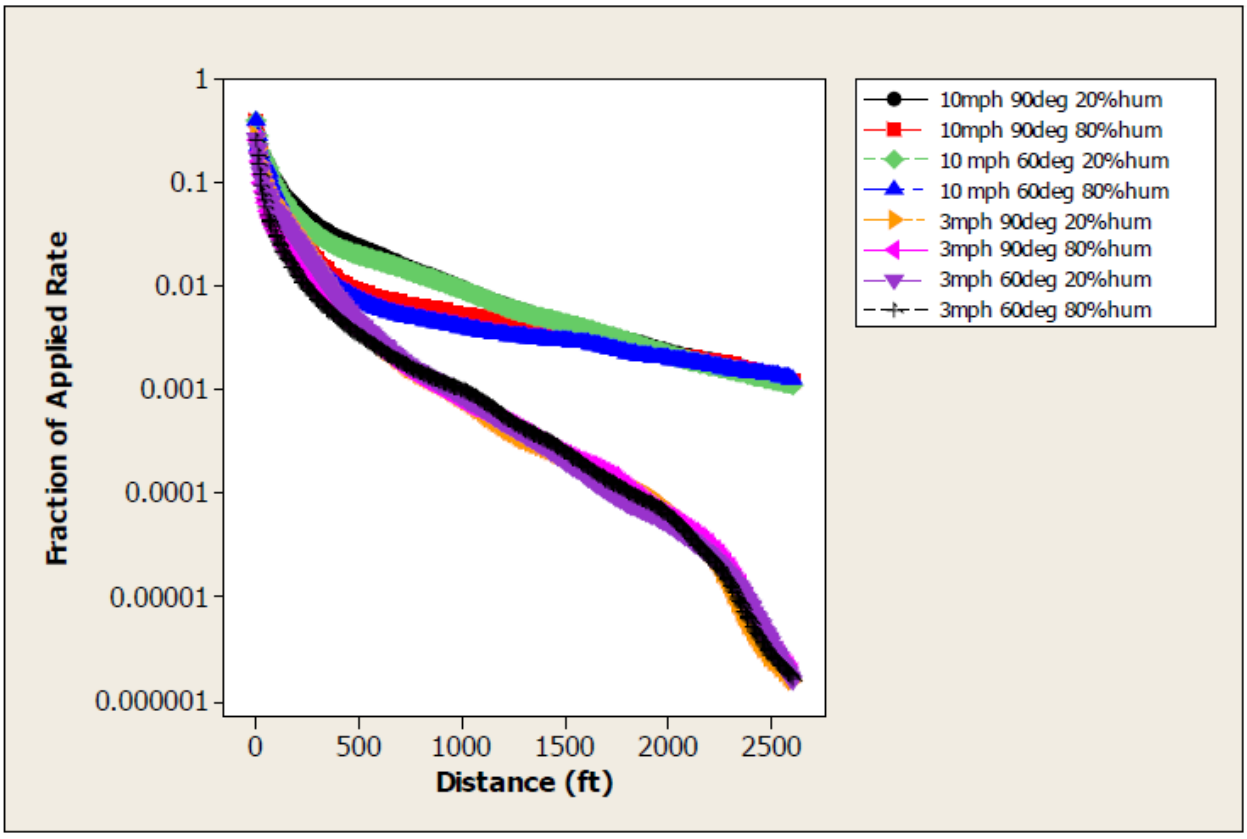
Aircraft	Distance to 0.35% of application rate (ft)	Air Speed (mph)	Aircraft Weight (lbs)	Semi-span or Rotor Radius (ft)	Number of Nozzles
Fixed Wing					
AT802A	1174	145	11160	29	39
AT401	1122	120	6000	24.5	42
Trush	1102	140	7665	23.75	32
AT502	1096	155	6660	25	34
AT301	1037	120	5600	22.6	30
AgCat*	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 aircraft. 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 air blast and ground will be considered separately.

Orchard Airblast. The AgDRIFT orchard air blast empirical model outputs the value of the empirical function. In the case of the least squares fit empirical function this values 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 specific California scenario. The AgDRIFT user manual does not state why a 90th percentile is not estimated for orchards. At the 1999 SAP OPP staff did present tolerance bounds for orchard air blast (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 values 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 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 x 320 ft) this is 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

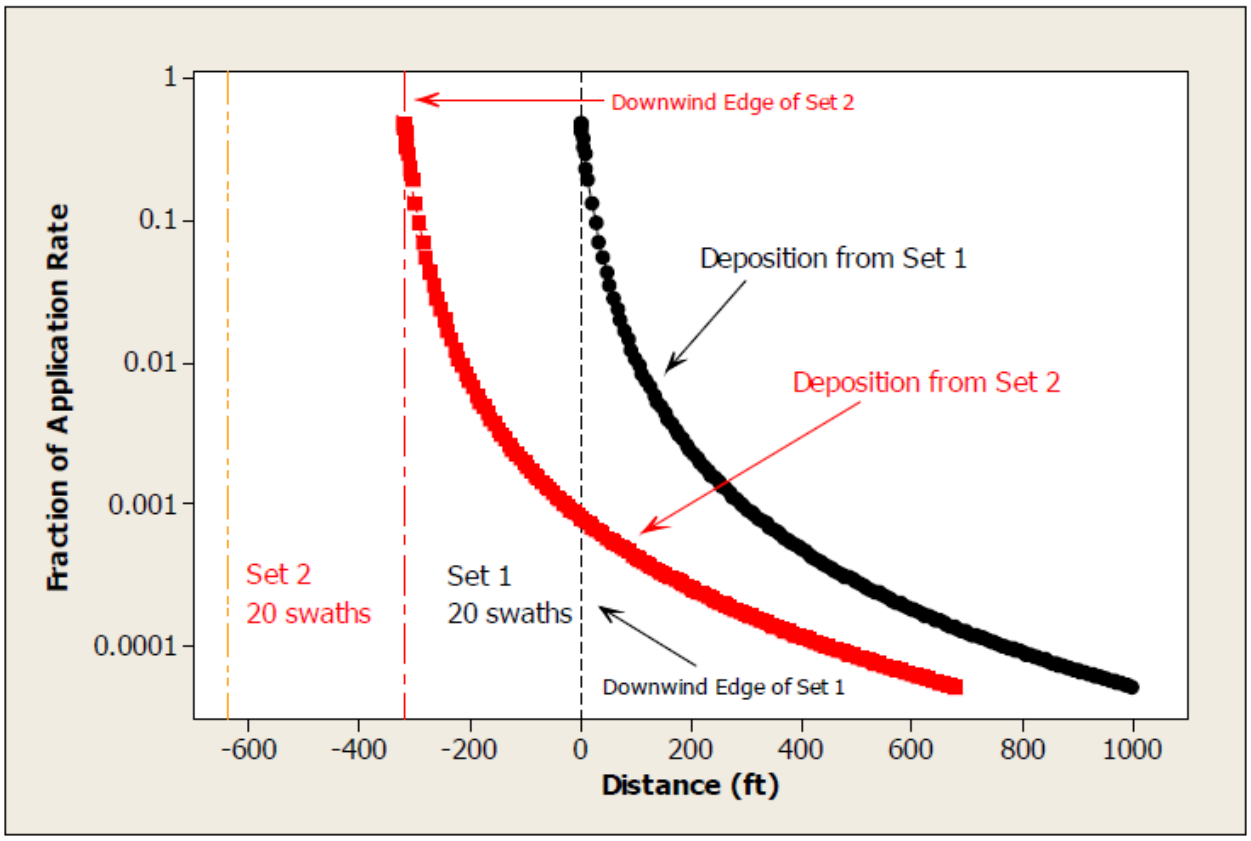
Table 6. Ground boom. Each set of 20 swaths is 900 ft wide. Downwind deposition curves are offset by the appropriate number of feet and then overlaid. When overlaying, upwind deposition curves are allowed to drop to zero at the model domain limit of 997.4 ft.

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.



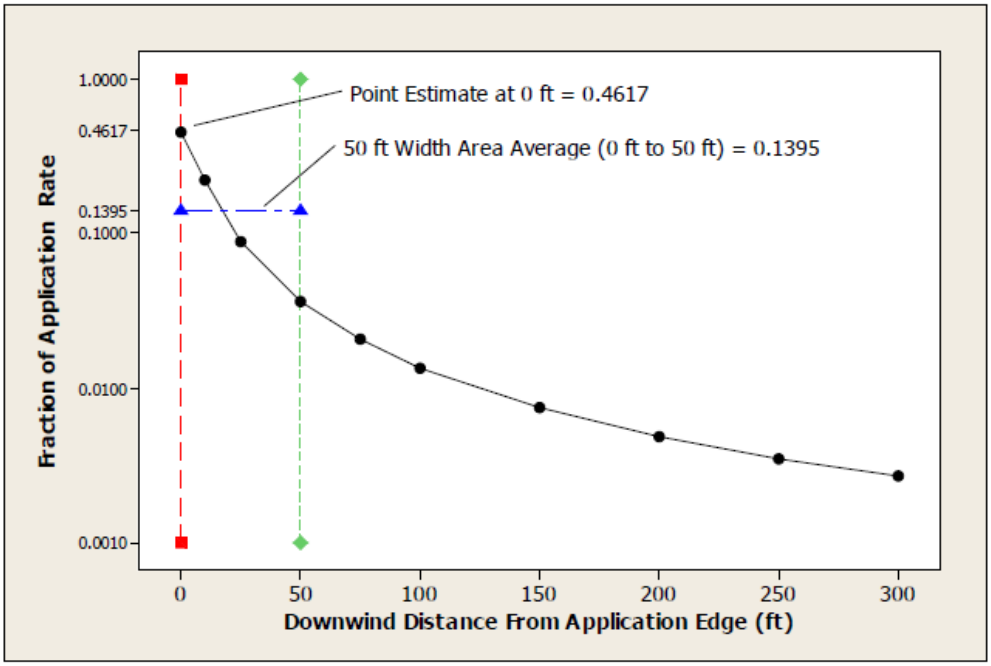
As stated above, this procedure was only implemented if the resulting deposition from the offset upwind swaths was within the prediction domain of the model. The aerial algorithm estimates deposition up to 2605 ft directly downwind of the application (the far field Gaussian handoff was not used in this 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. While these assumed empirical functions may be correct, there is no way 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 those estimates. 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 slowing and more likely over estimate deposition rather than underestimate. See the AgDRIFT manual for 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 6 to 8), dormant apples (Tables 9 to 11), and grapevines (Tables 12 and 13) 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 6. 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 7. 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 8. 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 9. 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 10. 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 11. 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

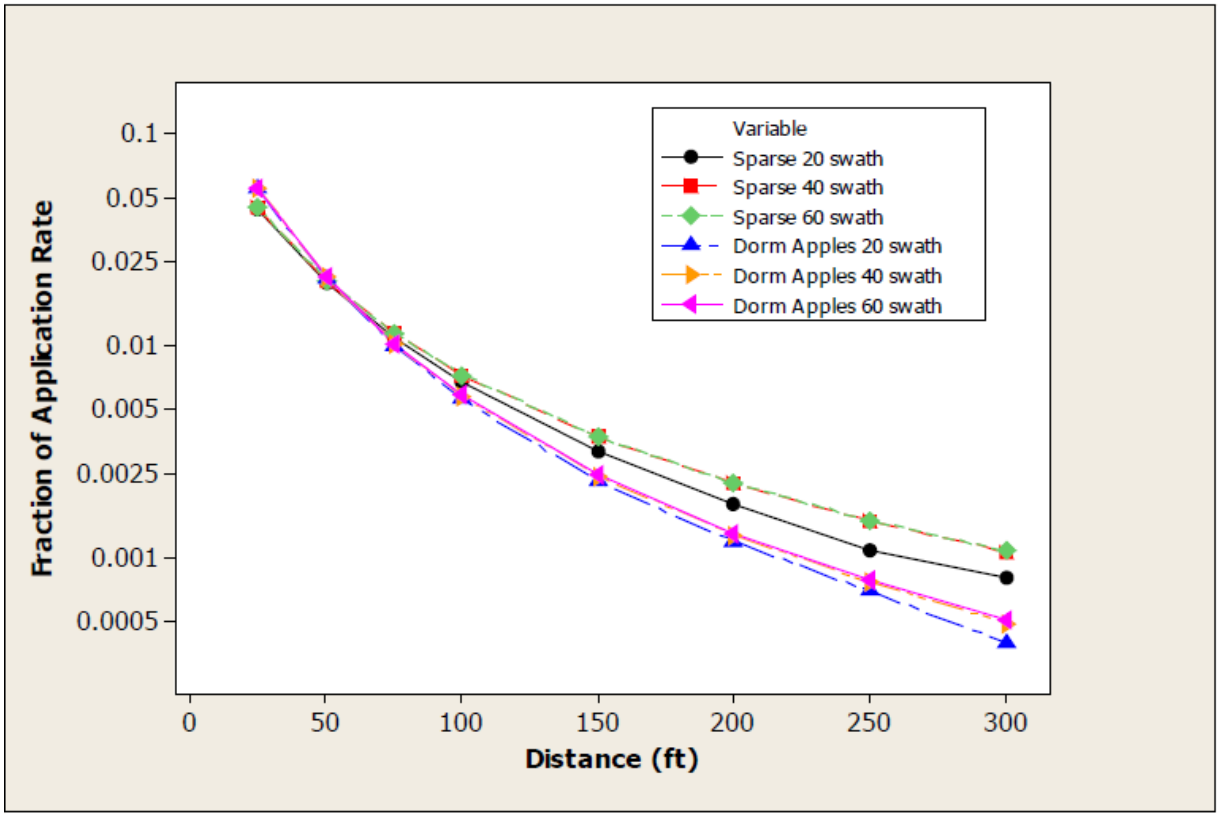
Table 12. Grape vineyard conventional sprayer 20 swath 50th percentile deposition estimates. The development procedure for these deposition estimates is described in the text.

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 13. Grape 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 14 and 15) and high boom (Tables 16 and 17) 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 14. 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 15. 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

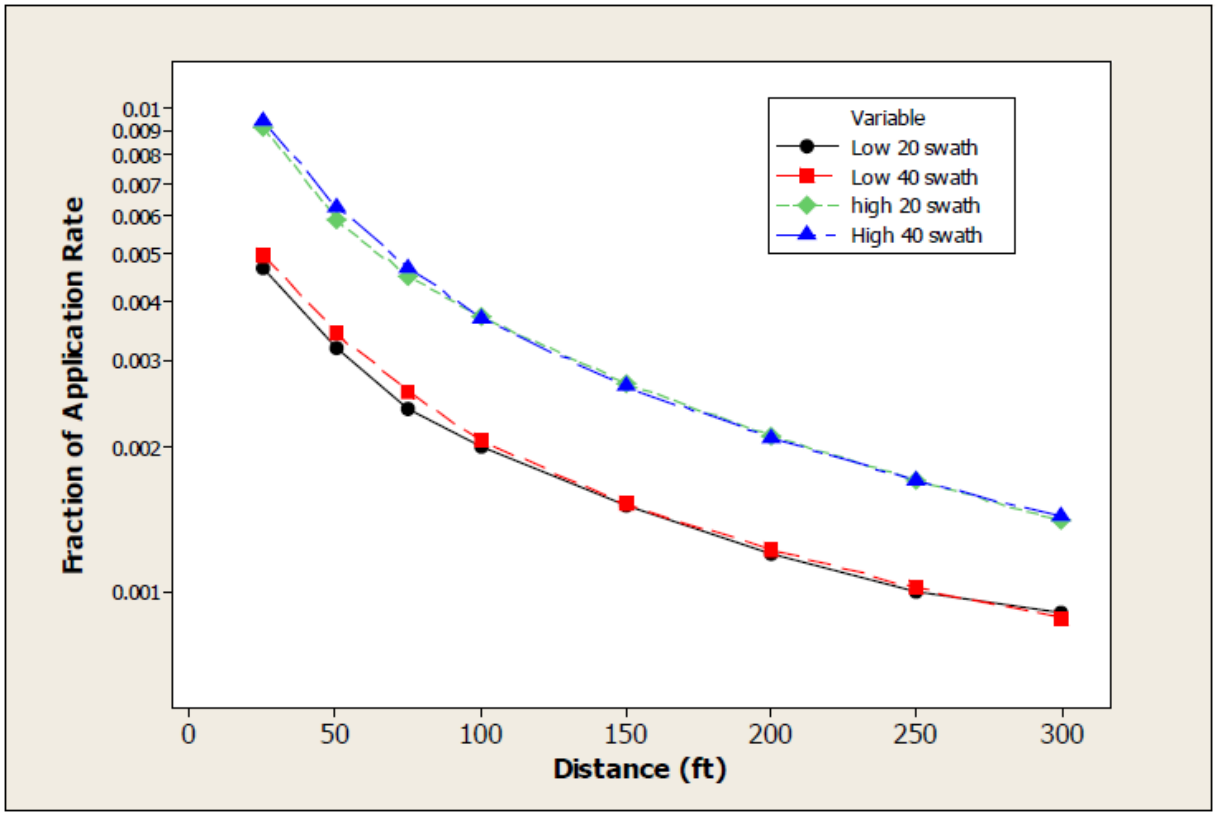
Table 16. Ground boom deposition. High boom and medium/coarse spray quality 20 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

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 17. 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 18 and 19. 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.

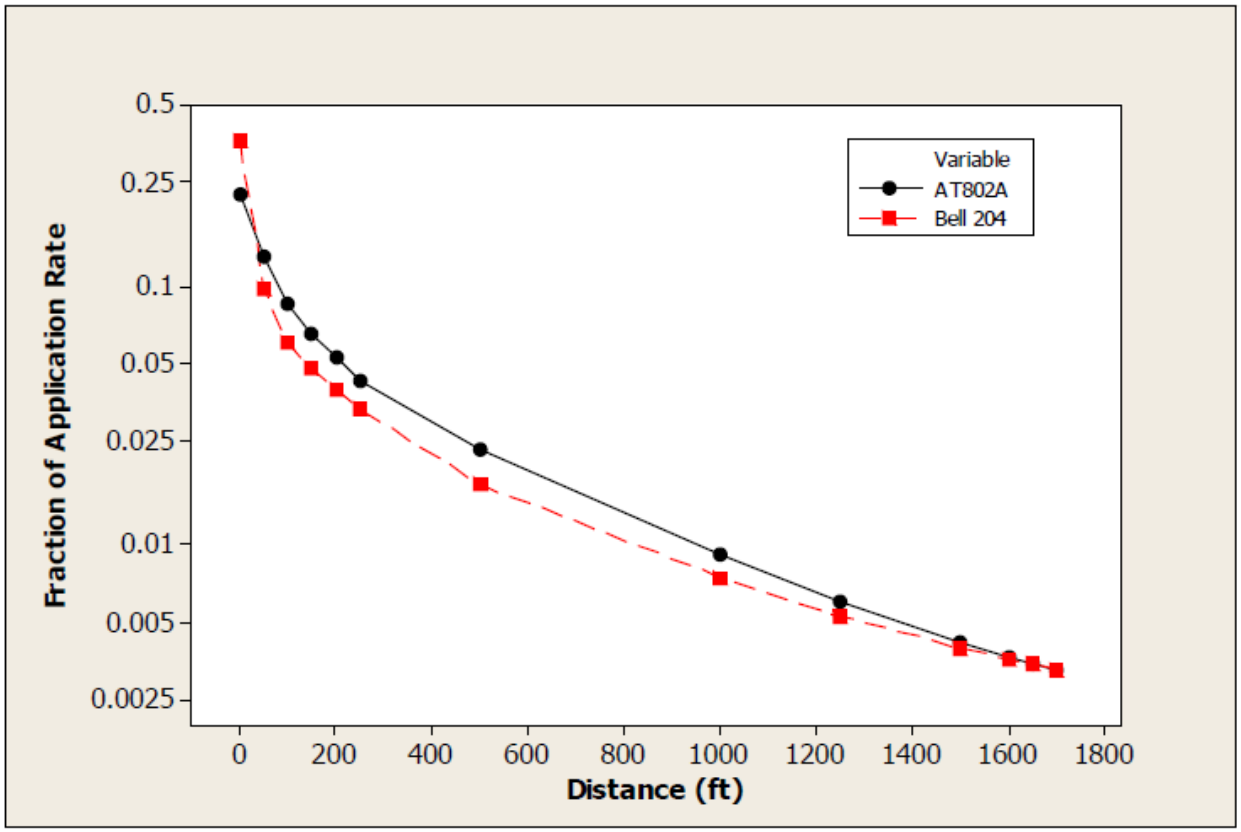
Table 18. Fixed-wing aerial application deposition. AT802A medium spray quality 50 swath 50th percentile. The development procedure for these deposition estimates is described in the text.

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 19. 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

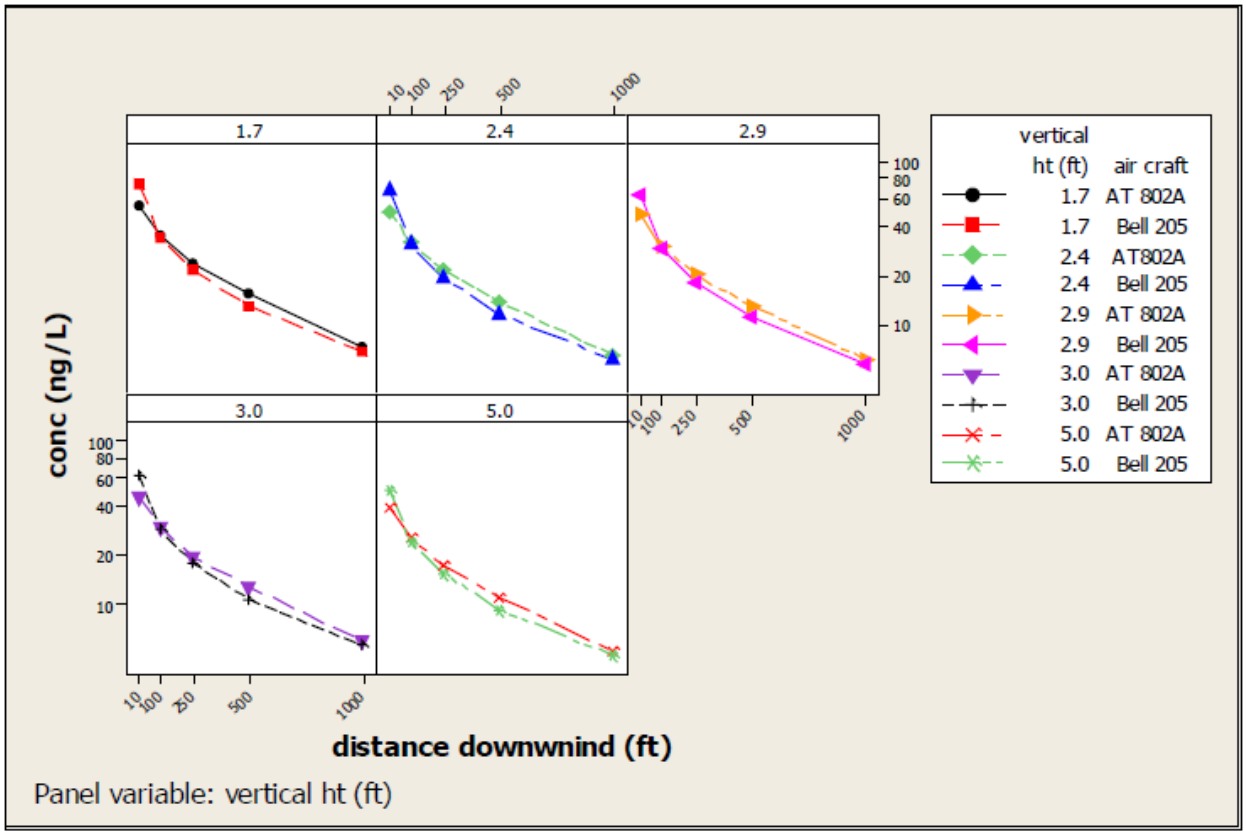
ground level are shown in Table 20. Figure 7 shows the change in 1-hr TWA air concentration with height for the vertical planes between 10 ft and 1000 ft downwind of the application edge. At the minimum federal label buffer zone of 10 ft, for the breathing heights of toddlers to adults (1.7 ft and 5 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.

Table 20. Selected 1-hr time weighted average (TWA) air concentrations (ng/m^3) 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 is described in the text.

Height Above Ground		1-Hr TWA Air Concentration (ng/m^3)	
		Aircraft Model	
Inches	Feet	AT802A Fixed Wing	Bell 205 Helicopter
0	0	n/a[1]	n/a[1]
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] The AGDISP model does not estimate air concentrations at ground level.

Figure 7. One hour time weighted air concentrations (ng/m^3) 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)

Both fraction of the applied mass that is measured as horizontal deposition (and by extension, the mass measured as horizontal deposition) and air concentrations associate with a particular application are functions of the finished spray volume expressed as gallons per acre (GPA) and the active ingredient (ai) application rate (lb ai/ac). When comparing two scenarios of GPA and application rate, this relationship also changes with the distance downwind. Thus, the designation of a "reasonable worst case" scenario is not simple.

The application tank mix scenarios shown in Table 21 were simulated using AGDISP for both fixed wing (AT802) and rotary (Bell 205) aircraft. The same aircraft set-ups that have been used throughout the Chlorpyrifos spray drift analysis were used for this analysis. Only the tank mix was changed for each scenario. The base finished spray volume is designated as 2 GPA. This is consistent with 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 application rate is designated as 2 lb ai/ac. Thus, for this analysis the base tank mix is 2 GPA finished spray volume and 2 lb ai/ac. All other tank mix combinations will be compared to this base. The Cheminova NUFOS 4E insecticide chlorpyrifos formulation that has 4 lb ai/gallon (0.5 lb/pint) was used for this simulation. For this formulation the ai is 45% by volume. 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 21. Tank mix calculations for the AGDISP tank mix comparison runs.

2 GPA Finished Spray (16 pints)			
ai[1] rate per acre	formulation volume per acre	Proportion of tank mix that is ai	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%
4 lb	8 pints	8/16*0.45 = 0.225	23%
6 lb	12 pints	12/16*0.45 = 0.338	34%
15 GPA Finished Spray (120 pints)			
ai rate per acre	formulation volume per acre	Proportion of tank mix that is ai	Percent ai in the tank mix volume[3]
1 lb	2 pints	2/120*0.45 = 0.008	1%
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	2%
4 lb	8 pints	8/120*0.45 = 0.030	3%
6 lb	12 pints	12/120*0.45 = 0.045	4.5%

[1]Active ingredient
[2]Rounded up to the nearest 1%
[3]Rounded up to the nearest 0.5% rather than 1% because the ai percentage is much smaller

Figure 8 shows, for the AT802-A fixed-wing aircraft, a comparison of the tank mix scenarios with the base tank mix of 2GPA and 2 lb ai/ac. The curves in Figure 8 depict the result for each scenario normalized to the base tank mix (at each distance the scenario results is divided by the result for 2GPA and 2 lb/ac). All six plots are on the same scale. Thus, a comparison of changes in results with scenario and distance can be assessed. The horizontal deposition results are presented in two ways. First the fraction of application rate deposited for each tank mix scenario is shown. In this presentation format the direct effect of application rate on the horizontal deposition mass is not shown but the relative effects are emphasized. Second, deposition of the actual mass for each scenario is shown. In this presentation format the change in mass deposition with changing application rate is emphasized. The air concentration results use the actual air

concentrations (ng/L) only. Thus, the air concentration comparisons shown in Figure 8 incorporate directly the effect of changing application rate.

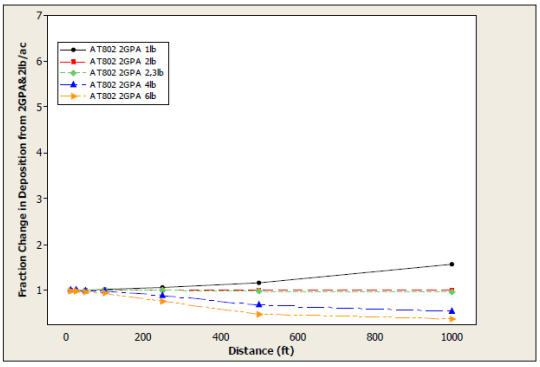
Across all combinations of finished spray volume and application rates, near field (within about 200 ft of the application edge) the horizontal deposition expressed as a fraction is reasonably similar (e.g., the fraction of application rate deposition ratio of base tank mix to scenario tank mix is close to 1.0) (Figure 8a and 8b). However, in the far field the change in fraction of application rate deposition ranges from about half the base rate for 2 gal/ac and 6 lb ai/ac to approximately double the base rate for all the 15 gal/ac scenarios. These results indicate that simple multiplication of a base fraction of application rate deposition curve does not produce the same results as if the AGDISP model (or AgDRIFT model) was run for each tank mix scenario.

Comparison of the mass of horizontal deposition using the 2 gal/ac and 2 lb ai/ac base tank mix shows that the relationship between application rate and deposition for both 2 gal/ac and 15 gal/ac finished spray is as expected between the field edge and about 100 ft downwind (figure 8c and 8d). However, further downwind, beyond 100 ft, the ratio between the base tank mix and the scenarios diverge from the straight multiples of 2 lb ai/ac. For the2 gal/ac scenarios, the ratio of the mass deposited to the base tank mix approaches 1.0 for all the application rates. For the 15 gal/ac scenarios the mass deposited increase in the far field to ratios between 1.0 and 5.3, depending upon the application rate. Air concentration ratios are shown in Figure 8e and 8f. Air concentration ratios for the 2 gal/ac application rates follow a trend similar to the mass deposited. However, the 15 gal/ac application rates show higher ratios with the base tank mix at the application edge and an increasing ratio with the base tank mix with distance downwind.

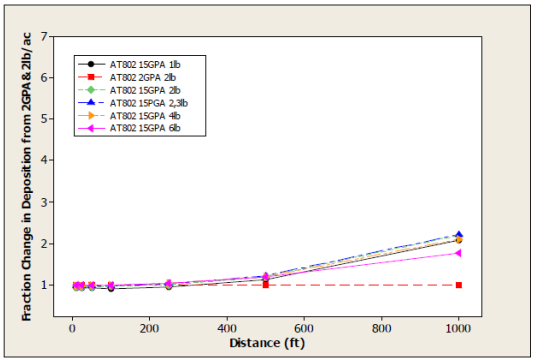
These results imply a tank mix effect that is not considered if the default inputs alone are used to produced horizontal deposition and air concentration estimates. The choice of 2 gal/ac finished spray volume may not be the most health protective scenario. The higher finished spray volume per acre appears to increase both deposition in the far field and increases air concentrations throughout the model domain.

Figure 8. Change in deposition and air concentrations with volume of finished spray (GPA), application rate (lb ai/ac), and distance (ft) for aerial applications with the AT802A fixed wing aircraft. The base scenario is AT802A aircraft 2GPA finished spray and 2 lb ai/ac application rate (AT802 2GPA 2lb).

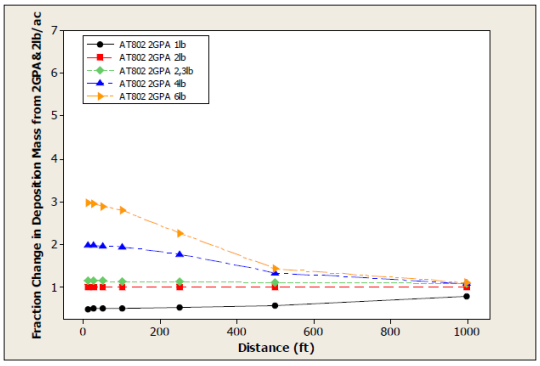
a. 2 GPA Horizontal Deposition Fraction



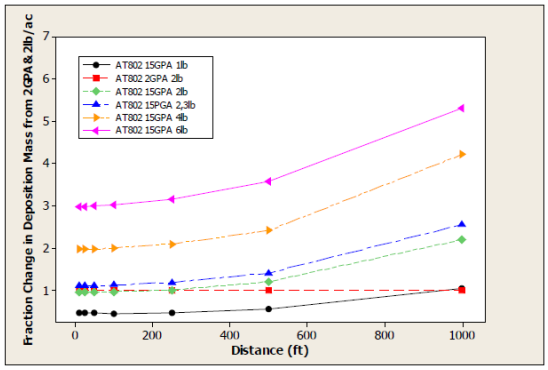
b. 15 GPA Horizontal Deposition Fraction



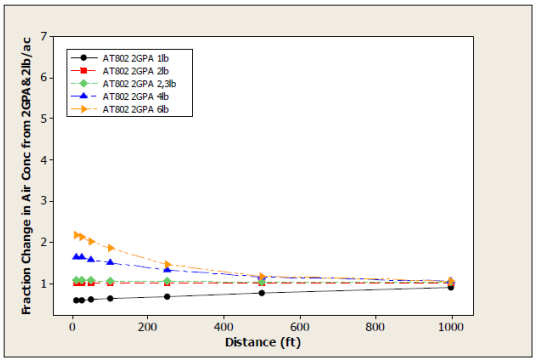
c. 2 GPA Horizontal Deposition Mass



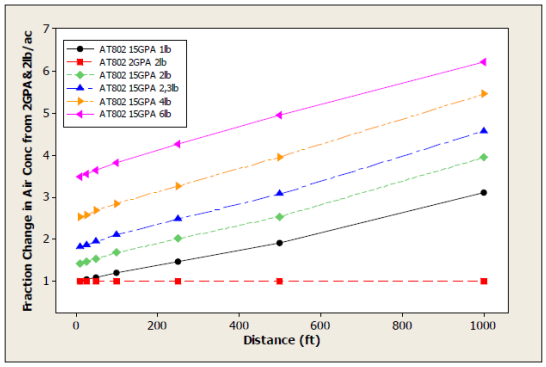
d. 15 GPA Horizontal Deposition Mass



e. 2 GPA Air Concentrations



f. 15 GPA Air Concentrations



Comparison with U.S. EPA Results

Both this analysis and the United States Environmental Protection Agency (USEPA) 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 were able to obtain 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 air blast 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 USEPA orchard airblast simulations used consistent inputs. The only differences are due to USEPA rounding up to 2 decimal places for the horizontal deposition. USEPA 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 22.

Table 22. 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 USEPA field edge horizontal deposition estimates are in error (Per. Comm. Charles Peck, USEPA. 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 23 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 23. 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]USEPA field edge deposition estimates are in error (Per. Comm. Charles Peck, USEPA. 2014).

Aerial. Differences between this analysis and USEPA for aerial simulation inputs produces differences in the horizontal deposition and air concentration estimates. The most important difference is that this analysis used AGDISP 8.28 (Teske, 2013) to simulate the aerial application scenarios while USEPA used AgDRIFT 2.1.1 regulatory version. For this comparison the USEPA Tier II modeling inputs will be compared. Table 24 follows the format of the AgDRIFT 2.0.05 user's manual (Teske, 2002). and shows the input comparisons for the fixed wing aircraft scenario. 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. AgDRIFT Tier I default inputs are shown in Table 24 for the AgDRIFT inputs that were not changed by USEPA from the defaults for the Tier II model runs.

Table 24. 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 and 15 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]USEPA 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.

Deposition estimates for 2 lb ai/ac application rate are compared in Table 25 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). 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. This effect is apparent in Figure 9. The horizontal deposition estimates reported in this analysis are higher relative to USEPA estimates 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 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. In addition, USEPA used simple multiplication of results from a single AgDRIFT run that produced horizontal deposition for a base application rate and finished spray of 2 GPA. This analysis indicates that simple multiplication of the horizontal deposition from a base application rate to adjust for desired application rates will not yield the same results as model runs for each of the desired application rates (Figure 10).The difference is small in the near field but increases in the far field. Because of this effect, this analysis 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. Figure 10 illustrates the effect on horizontal deposition for a spray volume of 2 gal/ac versus a spray volume of 15 gal/ac. As with application rate, the effect is largest in the far field (greater than 300 ft). This analysis included the spray volume analysis as part of the higher application rates scenarios, however, spray volume has an effect at all application rates.

Table 25. 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	This Analysis 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 9. Aerial application horizontal deposition estimates expressed as fraction of 2 lb ai/ac application rate as modeled by 5 different AgDRIFT and AGDISP scenarios.

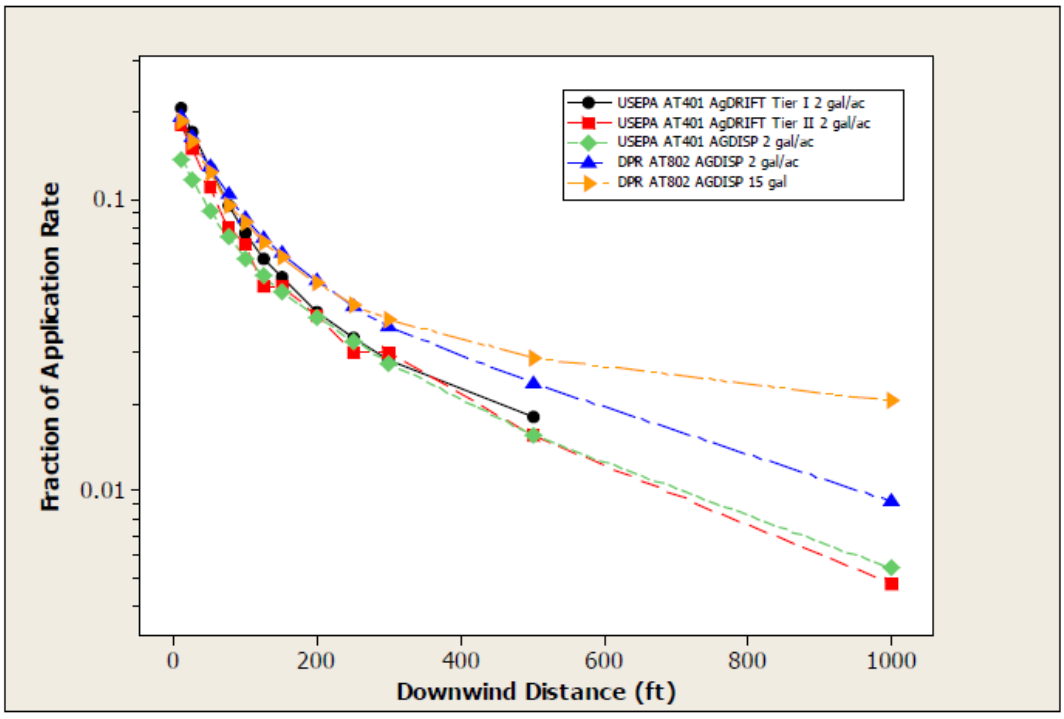
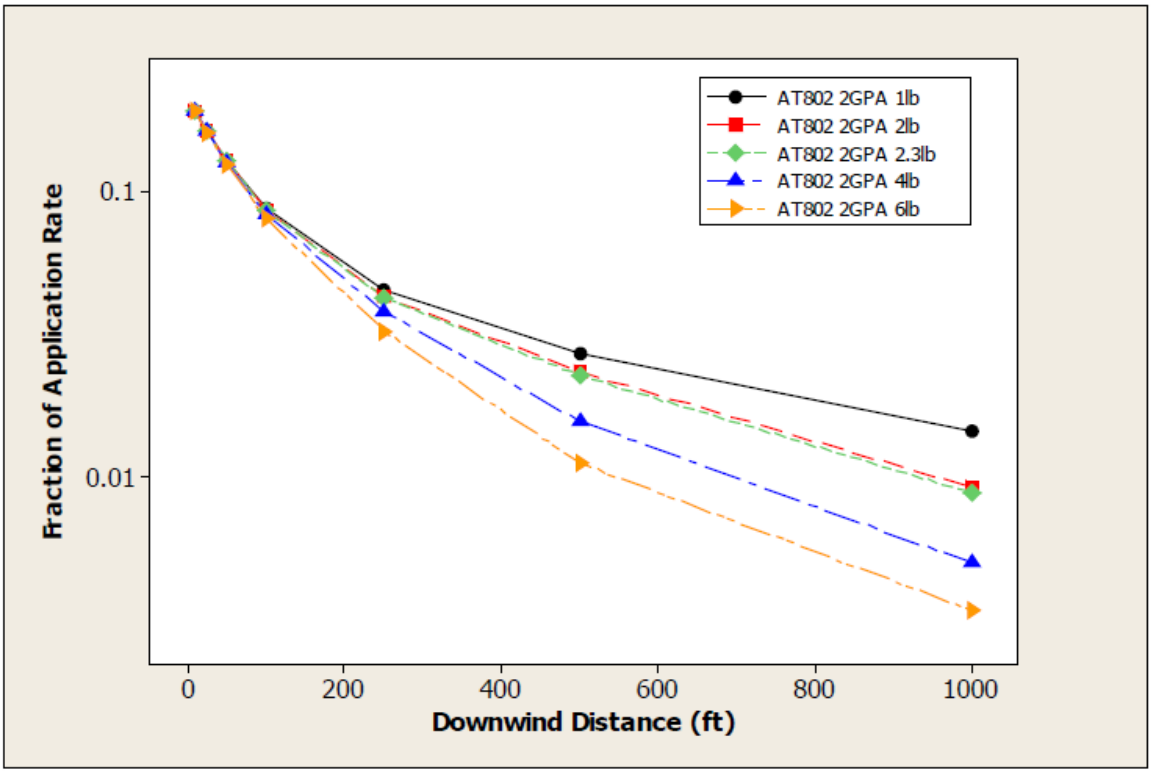


Figure 10. Effect of application rate on aerial application downwind horizontal deposition expresses as a fraction of application rate. The AT802A aircraft was used for these simulations. The simulation inputs are shown in Table 3.



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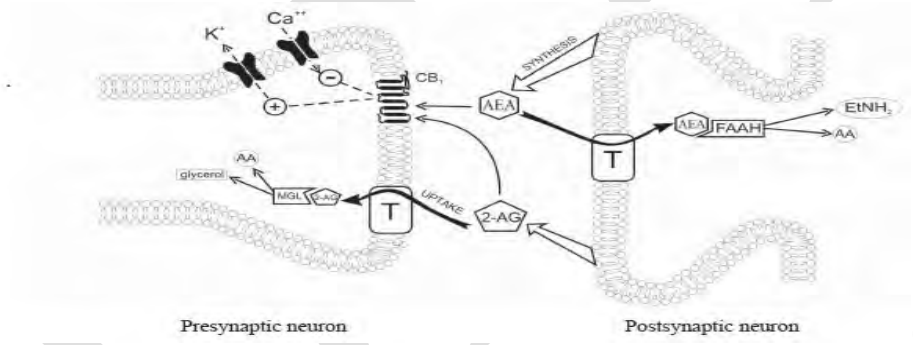
APPENDIX 4 BACKGROUND ON THE ENDOCANNABINOID, DOPAMINERGIC AND SEROTONERGIC SYSTEMS

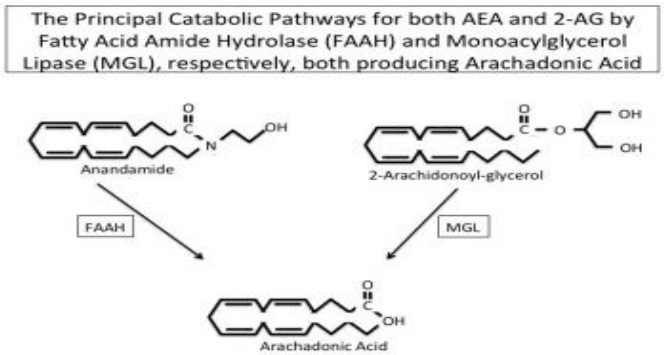
I. The Endocannabinoid System

The endocannabinoid system in the CNS consists of the arachidonic acid-based lipids: anandamide (*N*-arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG), both of which are ligands for the cannabinoid receptors (Pertwee 2006). Endocannabinoids are synthesized as needed and have short half-lives before being degraded by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Receptors for AEA and 2-AG are CB1 and CB2 G-protein membrane receptors (guanine nucleotide-binding proteins that transmit signals from stimuli outside a cell to the interior) (Melis and Pistis 2012; Ohno-Shosaku and Kano 2014; Pertwee 2008) (Figure 13).

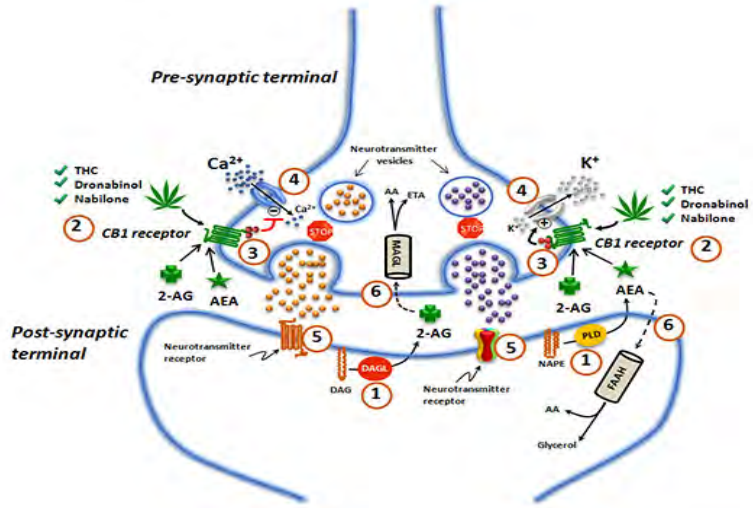
Endocannabinoid action requires pre-synaptic signals to release of neurotransmitters (e.g., ɣ-aminobutyric acid [GABA], glutamate) into the synaptic cleft, which then bind to receptors on the post-synaptic neural membrane to initiate a signal (excitation, inhibition, second messenger cascades). Depending on the signal, the sequence can result in the synthesis of AEA and/or 2-AG (expression is independent) stimulated by calcium ion (Ca^{++}) influx via voltage-sensitive calcium channels (Pertwee, 2008). Upon release into the synaptic cleft, the cannabinoids can be degraded by FAAH (AEA → arachidonic acid + ethanolamine) post-synaptically; or MAGL (MGL) (2-AG → arachidonic acid +glycerol) pre-synaptically with or without binding to CB1 or CB2 receptors (Brock 2005; Pazos et al. 2005; Yamaguchi et al. 2001) (Figure 14Figure 15).



(A)



(B)



(C)

Figure 14 Metabolism and Action of AEA and 2-AG: (A) and (C), respectively.

AEA and 2-AG release, interactions and metabolism pre- and post-synaptically. They are then broken down pre- and post-synaptically by FAAH and MAGL (i.e. MGL) (detailed in (B)), respectively after completing their action.

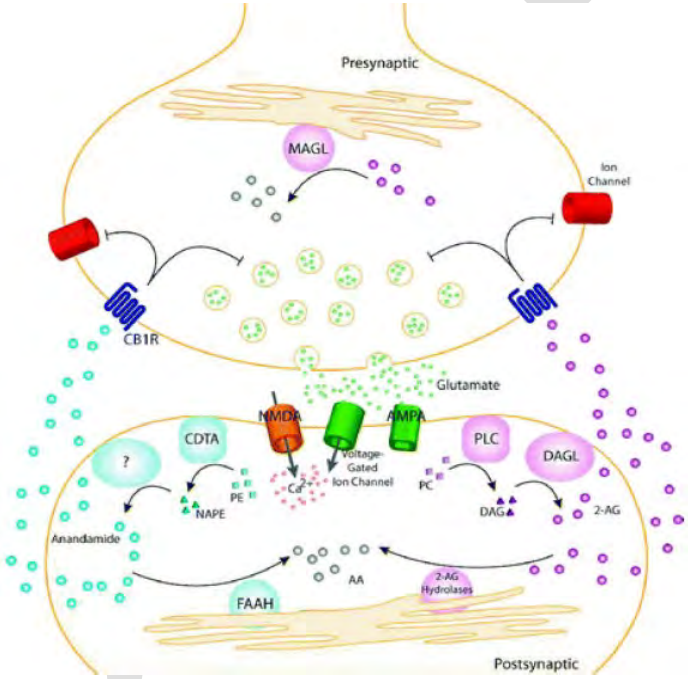


Figure 15 Endocannabinoid Signaling Pathway (Ahn et al., 2008).

Neurotransmitters are released (e.g., glutamate) and post-synaptic receptors (e.g., α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl- D-aspartic acid (NMDA)) and voltage-gated ion channels are activated, allowing influx of Ca^{2+} resulting in endocannabinoid synthesis. AEA is synthesized by a calcium-dependent transacylase (CDTA) and other unknown enzymes. 2-AG is synthesized by phospholipase C (PLC) and diacylglycerol lipase (DAGL). Endocannabinoids then migrate from postsynaptic neurons to CB1 receptors located on presynaptic neurons. CB1 receptors regulate ion channels and inhibit neurotransmitter release. Endocannabinoid signaling is then terminated by degradative enzymes. AEA is hydrolyzed to arachidonic acid by FAAH in the postsynaptic neuron. 2-AG is hydrolyzed to arachidonic acid primarily by MAGL in the presynaptic neuron.

V.B.2.b Endocannabinoid-Related Effects on Stress, Exploration, Social Behavior and Anxiety: Disruption of the endocannabinoid system and the resulting effects on social behavior, and stress is well-characterized in animal models (Haring et al. 2011; Jacob et al. 2009; Lafeneˆtre et al. 2009). The targets are glutamatergic and GABAergic cortical interneurons. Glutamate receptor inhibition leads to decreased object exploration, social interactions, and increased aggressive behavior in mice whereas inhibition of GABA receptors results in an increased exploration of objects, socialization, and open field movement (Haring et al. 2011).

V.B.2.c Endocannabinoids and Glucocorticoids: The endocannabinoid system is found throughout the cortico-limbic and hypothalamic systems that regulate the Hypothalamic-Pituitary-Adrenal (HPA) axis (Gorzalka et al. 2008; Hill and McEwen 2010). They can regulate both excitatory and inhibitory neurotransmitter secretion but the predominant effect of endocannabinoids is to limit the activation of the HPA axis (Hill et al. 2010a; Hill et al. 2010b). Glucocorticoids are normally secreted in response to stress but continuous release can lead to adverse effects. Endocannabinoids are involved in the habituation-response to recurring stress stimuli via the HPA axis. AEA continuously suppresses HPA axis activity; however under stress stimuli, the levels in the amygdala precipitously decline. Subsequently, axis inhibition is released; glucocorticoid hormone (e.g. corticosterone) is secreted (Hill et al. 2010a; Hill et al. 2010b) which then stimulates 2-AG synthesis to again suppress the HPA axis. Increased glucocorticoids also stimulate AEA synthesis amygdala which then feeds back to inhibit HPA axis activity (Hill and Tasker 2012) (Figure 16).

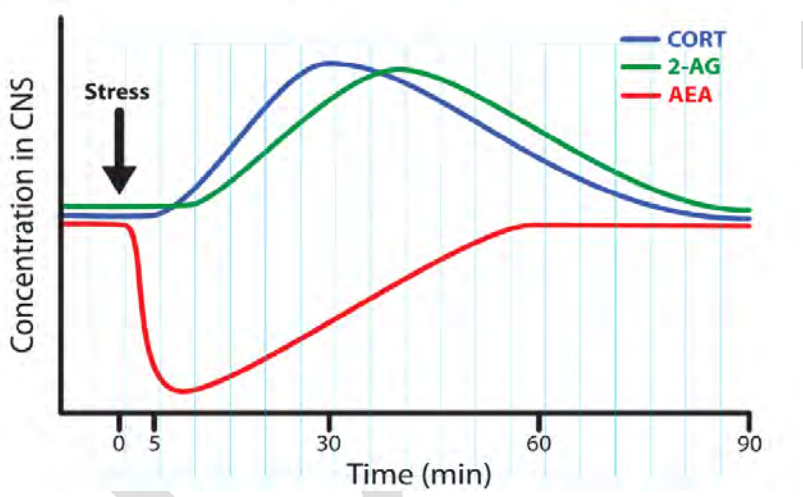


Figure 16 Cortisone, 2-AG and AEA Values Related to Stress Stimuli in Hill and Tasker (2012)

V.B.2.e Endocannabinoids and the Dopaminergic System:

Dopamine (DA) is a neurotransmitter that is synthesized in the CNS (Figure 17). Research in humans has shown an association between disrupted DA and depression (Galani and Rana, 2011). Animal studies have shown that dopaminergic system dysfunction leads to depression-related effects (Chaudhury et al., 2013). It is also established that dopamine and cannabinoids affect working memory, emotional learning and sensory perception (Chiu et al., 2010). Data have also shown that both the CB1 and dopamine receptors localize at the GABAergic synapses in the prefrontal cortex. Activation of either receptor suppresses GABA release and co-activation of both receptors by repetitive stimulation results in long-term

depression of inhibitory transmissions (LTD; Chiu et al., 2010). The figures below (Figure 17, Figure 18) adopted from Laviolette and Grace (2006) report the interactions between the endocannabinoid and the dopaminergic system. These systems are both involved with emotional processing and sensory perception.

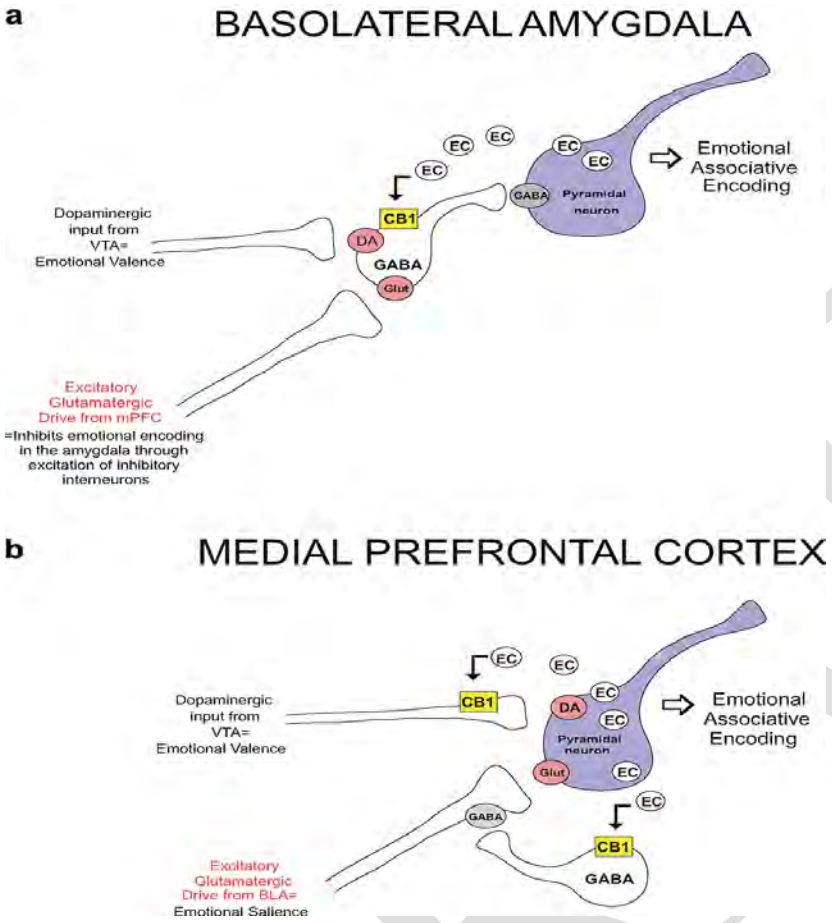


Figure 17 Illustration of the Associations between the Endocannabinoid and DA Signaling Substrates in the Basolateral Amygdala (BLA) and Medial Prefrontal Cortex (mPFC)

(a) In the BLA region, stimuli from the ventral tegmental area (VTA) and mPFC interact with inhibitory GABAergic interneurons. Excitatory input affects mPFC interneurons which then affect BLA pyramidal neurons by increasing GABA inhibition. In addition, DAergic input to mPFC interneurons can affect BLA pyramidal neuron activities by stimulating inhibitory DA receptors on the interneurons resulting in GABA inhibition. Endogenous cannabinoids released from amygdala pyramidal neurons can increase activity of pyramidal neurons by retrograde activation of inhibitory CB1 receptors located on the interneuron population. (b) In the mPFC, DAergic input from the VTA is under the regulation of CB1 receptors located on presynaptic DA terminals. Activation of this CB1 receptor population via retrograde endocannabinoid release from native cortical neurons can in turn inhibit DA input to cortical interneurons, thereby removing inhibitory DAergic input to cortical neurons and can also act through CB1 receptors located on inhibitory GABAergic interneurons that in turn decrease inhibition on presynaptic excitatory inputs to cortical pyramidal neurons leading to a net increase in cortical pyramidal neuron excitability (Laviolette and Grace 2006).

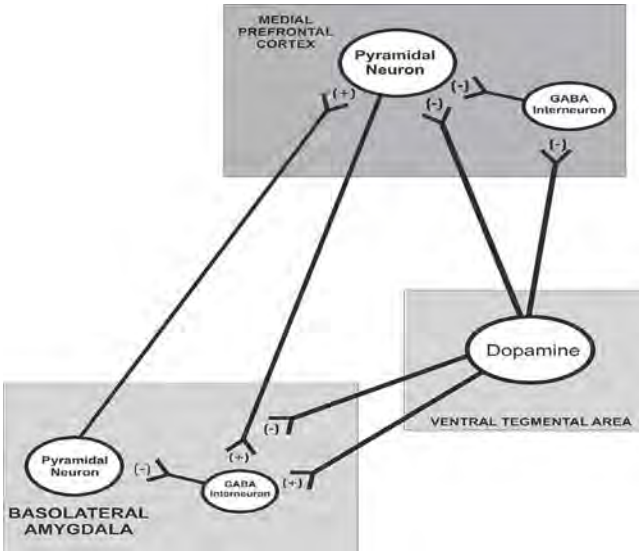


Figure 18 Simplified Scheme showing some of the Functional and Anatomical Connectivity Between the BLA, mPFC and VTA in Rodent Brain:
(+) symbolizes an excitatory postsynaptic effect whereas (–) symbolizes an inhibitory postsynaptic effect. The BLA both sends and receives excitatory glutamate projections to and from the mPFC. Electrical stimulation of the BLA can excite mPFC pyramidal neurons. Stimulation of the mPFC excites interneurons within the BLA and modulates emotional learning in neurons of the amygdala by activating inhibitory GABAergic interneurons, thereby inhibiting pyramidal output neurons of the BLA. In addition, functional input from the amygdala is required for emotional associative learning to occur in neurons of the mPFC the previously described mPFC neurons are involved in both the acquisition and encoding and extinction of emotionally-salient, conditioned associations. DAergic neurons of the VTA send and receive projections from both the BLA and mPFC (for simplicity only VTA DAergic outputs are shown). Dopamine input to the BLA modulates neuronal learning processes in BLA neurons and can excite amygdalar pyramidal neurons by inhibiting presynaptic excitatory, presumably glutamatergic input to these interneurons. Recent evidence also demonstrates that the VTA DA input to the BLA can directly excite the GABAergic interneurons. Dopamine transmission in both the BLA and mPFC is required for emotional-associative learning, demonstrating that DAergic input from the VTA to both of these regions can modulate emotional learning processes (Laviolette and Grace, 2006).

V.B.2.f CPF-Related Disruption of the Endocannaboid System Affects the Dopaminergic Signaling Pathways

Mohammed et al. (2015) has demonstrated that endocannabinoid-related CPF effects that result in emotional or social/behavioral abnormalities can also affect the monoamine signaling (dopamine signaling pathways) in the amygdala (Gardner, 2005). A preliminary study by Mohammed et al. (2015), using the same protocol as Carr et al. (2015) showed that preweanling rats treated with CPF at 0.5 mg/kg/d PND 10-16 showed decreased serotonin (5HT) and norepinephrine (NE) in rats as well as effects on dopamine metabolism. 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were increased at 0.5 mg/kg/d CPF and time to emergence into an illuminated area was decreased at 0.5 mg/kg/d. These metabolites are regulated by release of GABA and glutamate which are controlled by endocannabinoids in the brain. Increases in these metabolites in the amygdala were accompanied by decreased reactivity to new environments (related to emotions/anxiety/risk-taking behavior; Haring et al., 2001). The data in this study are preliminary but indicate, along with Carr et al. (2014, 2015) indicates that there are non-cholinergic effects occurring at doses below those that induce AChE inhibition in brain.

V.B.2.g. Serotonergic System and Adenylyl Cyclase

5HT is a neurotransmitter in the CNS that affects mood; feeling of well-being and cognitive functions (learning and memory) (Figure 19, Figure 20). It is also instrumental in the process of cellular

differentiation and organization of the brain during development (Aldridge et al., 2003). Disruption of the serotonergic system may lead to adverse effects to memory, learning, anxiety, depression and aggression (Chojnacka-Wójcik et al., 1991; Ogren et al., 2008; Meltzer et al., 2008; Spreitzer, 2008; de Boer and Koolhaas, 2005; Olivier et al, 1990; Winstanley et al., 2005).

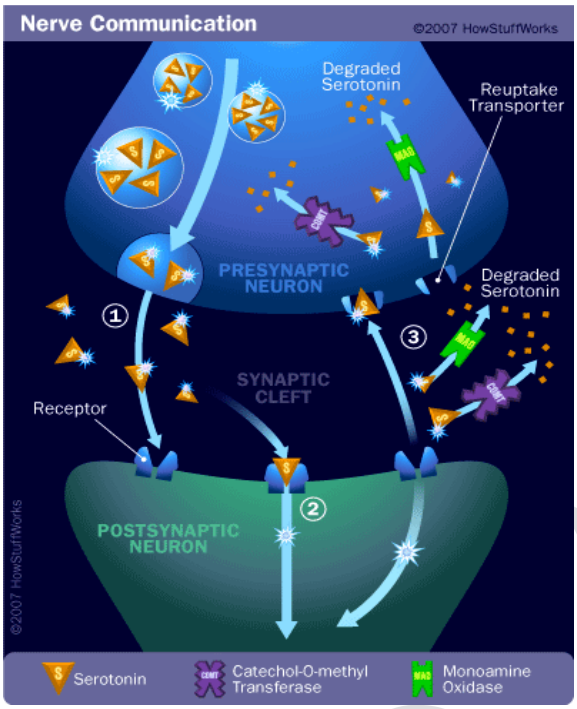


Figure 19 Adenylyl Cyclase (AC) Mechanism

AC is a regulatory enzyme that is soluble as well as membrane-bound in most cells. AC synthesizes cyclic adenosine monophosphate (cAMP) from cellular adenosine triphosphate (ATP) which then (functioning as a second messenger) relays the extracellular signals to intracellular receptors (Buck et al. 1999)**.** This enzyme also interacts with the dopaminergic system as shown below in Figure 18 (Winstanley et al. 2005)

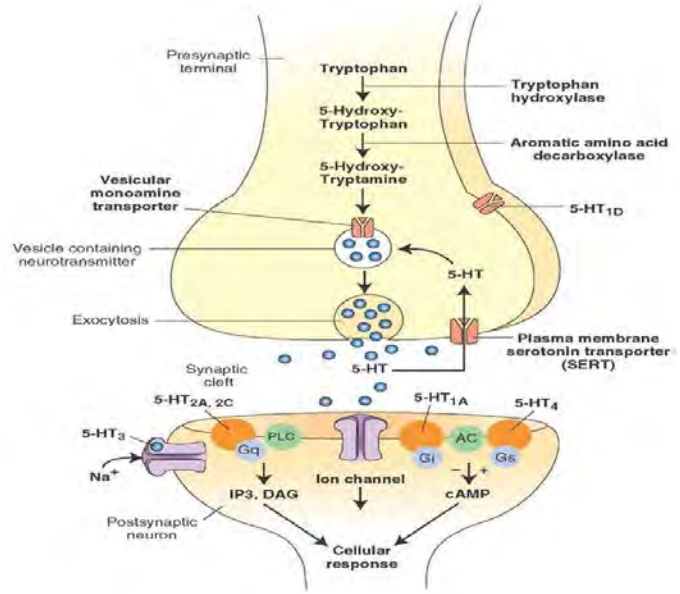


Figure 20 AC Interactions within the Dopaminergic System