

**EVIDENCE ON THE
DEVELOPMENTAL AND
REPRODUCTIVE TOXICITY OF
Chlorpyrifos (CAS # 2921-88-2)**

**REPRODUCTIVE AND CANCER HAZARD
ASSESSMENT SECTION
OFFICE OF ENVIRONMENTAL HEALTH HAZARD
ASSESSMENT
NOVEMBER 20, 2008**



CHLORPYRIFOS

- **Effective broad-spectrum organophosphate insecticide**
- **Currently has a variety of agricultural uses**
- **Exposure**
 - food and water
 - spray drift (proximity to field)
 - handling (workers)
- **Residential use cancelled in 2000**



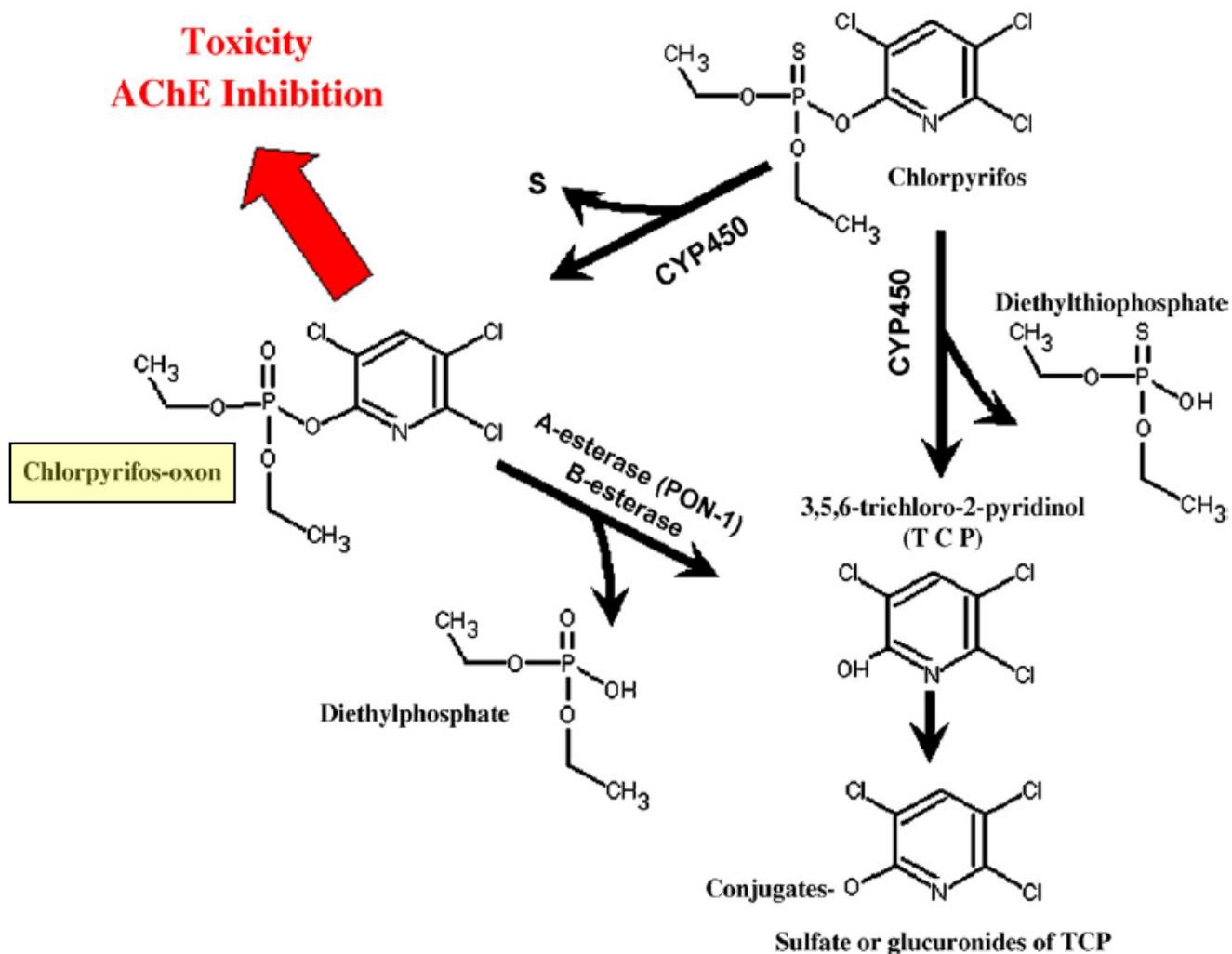
PHARMACOKINETICS

- **Absorption of Chlorpyrifos (CPF) varies with species**
 - **In humans, about 70% absorbed after oral exposure**
- **Rapidly metabolized**
- **Highest tissue concentrations found in the liver and kidney, but did not bioconcentrate; Fetal brain metabolite concentration twice as high as maternal brain concentration**
- **Mean half-life ($t_{1/2}$) in humans - 26.9 hours**
- **Excretion in Urine:**
 - Human - No parent compound detected; Metabolites in urine = TCP (62%) and DEP (40%)**



METABOLISM SCHEME FOR CHLORPYRIFOS

(Reproduced from Timchalk et al, 2005)



PARAOXONASE (PON1)

- A-esterase enzyme that hydrolyzes the active CPF metabolite (oxon)
- Polymorphisms of the PON1 gene influence both level of expression and its catalytic ability - determines the rate at which CPF is detoxified
- PON1 activity differs between ethnic groups - low vs high metabolizers
- High inter-individual variability, 131-164 fold range in PON1 status in a Mexican-American population
- PON1 levels shown to be lower at birth and in the young



NON - DART EFFECTS

- **Acute : Rat oral LD50 = 82–155 mg/kg**
- **Subchronic and Chronic:**
 - ↓ **plasma and RBC cholinesterase and brain cholinesterase in rats, dogs and humans**
- **Most sensitive effect is ↓ plasma, RBC and brain cholinesterase in the range of 0.03 to 3 mg/kg-day and above**
- **In animals significant ↓ plasma and RBC cholinesterase (ChE) occur at doses below those that cause ↓ brain ChE**



GENETIC AND RELATED EFFECTS ON CHLORPYRIFOS

- **Largely negative for mutagenicity and other short term assays for genotoxicity**
- **Some DNA damage in lymphocytes of mice using the comet assay**



CHOLINESTERASE INHIBITION: PRESUMED MODE OF ACTION FOR TOXIC EFFECTS

- **Sensitivity varies across species with Dog>Rat>Mice**
- **In humans plasma ChE is mainly BuChE which is more sensitive to ↓ than AChE**



RELEVANT METABOLIC CHANGES DURING PREGNANCY

- **Female rats, particularly pregnant rats, appear to be more sensitive than adult male rats to cholinesterase inhibition**
- **Pregnant female rats had lower plasma, brain, and liver carboxylesterase activity than non pregnant**
- **PON 1 activity (i.e., detoxification) is reduced during late gestation (to 76%) in women**



CHLORPYRIFOS: ANIMAL DATA

Some reported developmental or reproductive toxicity endpoints such as

resorptions, ↓pup weight, ↑pup mortality

long-term effects on brain and behavior



STUDIES CONDUCTED PER REGULATORY GUIDELINES

Breslin 1991 (Breslin 1996)

Developmental Toxicity Study

- Pregnant Fischer 344 rats 0 (corn oil vehicle), 0.1, 3.0, or 15 mg CPF/kg-day, by gavage on GD 6 – 15

Two-generation Reproduction Study

- 30 M and F SD rats parental (F1 & F2) generations given CPF at 0, 0.1, 1.0, or 5.0 mg /kg-day in diet



Developmental Toxicity Study

- **↓ plasma and RBC ChE levels in dams**
- **no developmental effects in offspring**

Two-generation Reproduction Study

- **Significant ↓brain ChE of dams at 5 mg/kg-day**
- **↓Pup body weight (Day 4, Day 21) and
↑Pup mortality (Day 14, Day 21) at 5mg/kg-day
in the F1 litters only**
- **Neonatal effects observed in the F1 generation
not noted in the F2 generation**
- **No effects on histopathology of reproductive
tissues at any dose level**



Developmental Neurotoxicity (DNT) Study

Hoberman 1998

Maurrisen et al., 2000

Chlorpyrifos via oral gavage to (SD) pregnant rats (25 dams/group), on GD 6-PND11 at 0, 0.3, 1 or 5 mg/kg-day

- ↑ mortality soon after birth, in pups from high-dose
- gained weight more slowly than controls
- several indications of slightly delayed maturation

Authors noted:

High variability in the data

No overt effects in either dams or pups at 1 or 0.3 mg/kg/day

Maternal and developmental toxicity at the 5 mg/kg-day but not selective developmental neurotoxicity



UPON CLOSER REVIEW

**Adverse findings in the adult offspring (PND 66)
and consistent with PND12 results**

- alterations in motor activity, auditory startle response**
- brain structure (decreased measurements of the parietal cortex and hippocampal gyrus in the absence of significant brain weight deficits) at 1 and 5 mg/kg-day**



BRAIN MORPHOMETRY AT PND 66

Dimensions in μm of Specified Cross-sections of F1 female pups (n=6)	Dose (mg/kg-day)					
	0		1		5	
Parietal Cortex	1792	36.1	1716	36.4*	1700	55.6*
Hippocampal Gyrus	1708	57.6	1644	129.5	1592	86.8
Brain weight (g)	2.103	0.071	2.127	0.079	2.048	0.050

* Statistically significant, $p < 0.05$ Dunnett's test; Mean S.D



EFFECTS ON THE DEVELOPING NERVOUS SYSTEM

- **Lowest tested dose tested = 0.3 mg/kg-day**
**No behavioral effects observed in the offspring
at this dose level**
- **Brain morphometric changes at 1 mg/kg-day
and above. No data available at 0.3 mg/kg-
day**



EFFECTS ON NEURODEVELOPMENT

- Adults have persistent behavioral effects following gestational and/or early postnatal exposure in both rats and mice
- behavioral abnormalities emerge during adolescence and adulthood

- Slotkin/Levin and Calamandrei laboratories



OTHER RELEVANT ISSUES

- **Use of DMSO as a vehicle for Chlorpyrifos administration in animal studies**
- **Differences between developing animals and adults**
- **Distribution and metabolism in pregnant females and conceptuses**
- **Possible mechanism(s) of developmental toxicity**



USE OF DMSO AS A VEHICLE FOR CHLORPYRIFOS ADMINISTRATION IN ANIMAL STUDIES

- **Concordance between studies that used DMSO as the vehicle and those that did not use DMSO**
- **C_{max}, AUC and t^{1/2} values similar to bolus oral exposure in a milk vehicle**



DIFFERENCES BETWEEN DEVELOPING ANIMALS AND ADULTS

- Developing animals appear more susceptible to acute toxic effects of CPF than adults because of lower levels of detoxifying enzymes such as carboxylesterases (CbxE), and A-esterases**
- ↑Fetal brain ChE activity during GD 14-18 in control animals**
- Maternal brain ChE ↓ more than fetal brain ChE only in a repeated dosing regimen**



DISTRIBUTION AND METABOLISM IN PREGNANT FEMALES AND CONCEPTUSES

- **Dosage to nursing pups much reduced compared to the dams**
- **At 5 mg/kg-day a nursing pup's exposure was ~ 0.5 mg CPF/kg-day**
- **In spite of exposure via milk, the ChE levels of all tissues of high-dosage pups rapidly returned to near control levels by PND 5**
- **Alternate mechanisms to ChE inhibition?**



MECHANISM(S) OF DEVELOPMENTAL TOXICITY

Effects on the developing brain

- cell division.
- RNA synthesis during differentiation.
- cell signaling.
- nuclear transcription factors and cell differentiation.
- cholinergic synaptic function
- on catecholamine system
- oxidative stress
- gliogenesis and axonogenesis.



COMPARISONS OF PERSISTENT MAIN TREATMENT EFFECTS OF CPF WHEN ADMINISTERED IN DIFFERENT CRITICAL PERIODS (compiled from several studies)

Measure	GD 9-12	GD 17-20	PND 1-4	PND 11-14
ChAT	↑	(↓)	↓	↓
HC-3 binding	↓	↓↓	↓↓↓	↓
m ₂ AChRs	↓		ND	ND
DNA concentration	↓	↓	ND	ND
DNA content	↓	(↓)	ND	ND
Total protein/DNA	↑	↑↑	ND	ND
Membrane/total protein	↓	↓↓	ND	ND
Regional targets for cholinergic effects	hippocampus striatum	hippocampus cerebral cortex	hippocampus	hippocampus striatum
Regional targets for cellular effects	hippocampus striatum cerebral cortex	hippocampus cerebral cortex	ND	ND

Arrows indicate direction and comparative magnitudes of effects.

Parentheses denote effects with a consistent direction that did not achieve statistical significance

± indicates no consistent direction of change; ND = no determination done.



FINDINGS FROM RELATED STUDIES WITH CHLORPYRIFOS

- **behavioral effects may be mediated via a non-cholinergic mode of action**
- **elicit delayed-onset alterations, disrupting the “program” for the emergence of cholinergic activity**
- **corroborated by behavioral deficits in cholinergic contributions to working and reference memory that emerge in adolescence and adulthood similar to that for nicotine**



EFFECTS ON THE FEMALE REPRODUCTIVE SYSTEM

- changes in offspring growth or milk quantity and quality may constitute a female-specific endpoint of reproductive toxicity
- presence of CPF in the milk can be considered as affecting lactation, an essential component of female reproduction
- reduced pup weights (Day 4, Day 21) and increased pup mortality (Day 14, Day 21) in F1 rats of two-generation study



EFFECTS ON MALE REPRODUCTIVE SYSTEM

- No effects on histopathology of reproductive tissues
- Severe testicular damage resulting in reduction in sperm count and fertility at much higher dose levels
- Typically, studies in laboratory animals focus on fertility or sperm abnormalities and do not examine DNA damage
- The findings of DNA damage noted in few of the studies conducted for evaluating genotoxicity may support the findings in the human studies

