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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLUTION PREVENTION

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: May 20, 2011

SUBJECT: Chlorpyrifos: Review of the Comparative Cholinesterase (including chlorpyrifos oxon), special acute inhalation study and immunotoxicity studies.

PC Code: 059101 (chlorpyrifos)
And 659101 (chlorpyrifos oxon)
Decision No.: 436952
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0055409
MRID No.: Three studies (see table below)

DP Barcode: D380055

Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.:
40 CFR: N/A

Ver.Apr.08

FROM: John Doherty
Risk Assessment Branch V
Health Effects Division 7509P

THROUGH: Jack Arthur
Branch Chief
Risk Assessment Branch V
Health Effects Division 7509P

TO: Yan Donovan
And
Tom Myers
Review Manager Team 52
Pesticide ReRegistration Division 7507P

I. CONCLUSIONS

The acute and repeat dose comparative cholinesterase study (2010, MRID 48139301) (including separate dosing with chlorpyrifos oxon), the special acute inhalation study (2010, MRID 48139303) with assessment of cholinesterase inhibition and the series 870.7800 immunotoxicity study (2010, MRID 48139304) with chlorpyrifos were reviewed and the conclusions of each study together with their classification are presented in the Table below.

Rec'd in OPP 5/26/2011

The comparative ChE and special acute inhalation studies were classified as Acceptable/Non-Guideline. The immunotoxicity study was classified as Acceptable/Guideline. The DERs are attached.

II. ACTION REQUESTED

Risk Assessment Branch V (RABV) was requested to review a special non-guidelne comparative cholinesterase (ChE) inhibition study in rat pups and adults, a special non-guideline acute inhalation toxicity study designed to assess RBC, plasma, lung and brain ChE in female rats and a guideline series 870.7800 immunotoxicity screen study in female rats.

III. RESULTS/DISCUSSION

A. *Comparative ChE study.*

The adults and pups were determined to have the same NOAELs for both chlorpyrifos and its oxon with the NOAELs for the oxon being lower than for chlorpyrifos except for the brain enzyme. Brain ChE was not inhibited by chlorpyrifos oxon in either pups or adults. Dosing pups in harvested rat milk or dosing adults by the dietary route for an acute dose with chlorpyrifos rendered the same NOAEL and LOAEL resulting from dosing in corn oil.

The pharmacokinetic data was considered of limited usefulness because of poor variability and limited number of assessments.

B. *Special Acute Inhalation Study with assessment of ChE inhibition and analysis of chlorpyrifos, chlorpyrifos oxon and TCP in the blood and urine.*

The study was determined to demonstrate both plasma and lung ChE inhibition at 3.7 mg/m^3, the lowest dose tested. RBC and brain ChE were inhibited at exposures levels of 12.9 mg/m^3 for blood and 53.5 mg/m^3 for brain ChE.

The analysis of blood for chlorpyrifos indicated the peak level at the end of the exposure period and declined rapidly after removal. The peak level for TCP in blood was 12 or 24 hours after removal from the exposure chamber. The pharmacokinetic data indicated that the two middle doses (12.9 and 22.1 mg/m^3) were nearly the same with respect to blood level of chlorpyrifos and TCP and urinary level of TCP. Thus correlation of blood levels of parent and metabolite with inhibition of ChE was limited.

C. *Series 870.7800 immunotoxicity study – rats.*

The immunotoxicity study was classified as Acceptable/Guideline and no indications of immunotoxicity were indicated.

D. ***Special report on benchmark dose modeling prepared by the Registrant's consultants (MRID 48139302) – no DER prepared and not comments from RABV at this time.***

MRID Summary Table Example

Study Type	MRID (year) Dose levels Classification	Comments (RBC and Brain ChE only)
Special Non- Guideline Comparative Cholinesterase inhibition study with both chlorpyrifos and chlorpyrifos oxon via gavage, milk (acute pups only), dietary (acute adults only) for both acute and repeat dosing. With analytical data for blood chlorpyrifos, chlorpyrifos oxon and the metabolite TCP.	MRID 48139301 (2010) Chlorpyrifos (acute dosing) : *Pups:* 0, 0.05, 0.1, 0.5, 2 or 5 mg/kg in corn oil or harvested rat milk. *Adults:* 0, 0.05, 0.1, 0.5 or 10 mg/kg in corn oil or dietary. Chlorpyrifos oxon (acute dosing): *Pups:* 0.005, 0.01, 0.05, 0.1 or 0.5, mg/kg in corn oil. *Adults:* 0, 0.01, 0.05, 0.1, 0.5 or 1 mg/kg in corn oil. Chlorpyrifos (repeat dosing): *Pups and adults:* 0, 0.05, 0.1, 0.5, 1, or 3.5 mg/kg/day for 11 days. Chlorpyrifos oxon (repeat dosing) *Pups and adults:* 0, 0.01 or 0.5. Classification: Acceptable/Non-Guideline.	Chlorpyrifos (acute dosing) : *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.5 and 2 mg/kg. *Brain AChE:* NOAEL is 2 mg/kg for both pups and adults. LOAEL is 5 mg/kg for pups and 10 mg/kg for adults. No difference between corn oil and rat milk or diet. Chlorpyrifos oxon (acute dosing): *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.1 and 0.5 mg/kg. *Brain ChE* – not inhibited. Chlorpyrifos (repeat dosing): *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.1 and 0.5 mg/kg/day. *Brain AChE:* NOAEL and LOAEL for both adults and pups are 0.5 and 1 mg/kg/day. Chlorpyrifos oxon (repeat dosing): *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.01 and 0.5 mg/kg/day. Note although the NOAEL and LOAELs are the same, there are quantitative differences at the LOAEL in some comparisons.
Special Acute inhalation study with ChE inhibition and pharmacokinetic data.	MRID 48139303 (2010) 0, 3.7, 12.9, 22.1 or 53,5 mg/m^3 nominal atmospheric concentrations. Achieved dosage : 0, 0.52, 2.86, 2.21 and 5.7 mg/kg. Acceptable/NonGuideline.	*Plasma and lung ChE:* LOAEL = 3.7 mg/m^3. NOAEL not established. *RBC ChE:* NOAEL = 3.7 mg/m^3. LOAEL = 12.9 mg/m^3. *Brain ChE:* NOAEL = 22.1 mg/m^3. LOAEL = 53.5 mg/m^3. No clinical signs.
870. 7800. 4-week dietary immunotoxicity in rats (females).	48139304 (2010) 0, 0.416, 2.13, 0r 10.7 mg/kg/day. Acceptable/Guideline	No evidence of immunotoxicity at any dose.
Special report on benchmark dose modeling prepared by the registrant's consultants.	48139302 (2010)	No DER prepared.

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MRID Summary Table Example

Study Type	MRID (year) Dose levels Classification	Comments (RBC and Brain ChE only)
Special Non- Guideline Comparative Cholinesterase inhibition study with both chlorpyrifos and chlorpyrifos oxon via gavage, milk (acute pups only), dietary (acute adults only) for both acute and repeat dosing. With analytical data for blood chlorpyrifos, chlorpyrifos oxon and the metabolite TCP.	MRID 48139301 (2010) Chlorpyrifos (acute dosing) : *Pups:* 0, 0.05, 0.1, 0.5, 2 or 5 mg/kg in corn oil or harvested rat milk. *Adults:* 0, 0.05, 0.1, 0.5 or 10 mg/kg in corn oil or dietary. Chlorpyrifos oxon (acute dosing): *Pups:* 0.005, 0.01, 0.05, 0.1 or 0.5, mg/kg in corn oil. *Adults:* 0, 0.01, 0.05, 0.1, 0.5 or 1 mg/kg in corn oil. Chlorpyrifos (repeat dosing): *Pups and adults:* 0, 0.05, 0.1, 0.5, 1, or 3.5 mg/kg/day for 11 days. Chlorpyrifos oxon (repeat dosing) *Pups and adults:* 0, 0.01 or 0.5. Classification: Acceptable/Non-Guideline.	Chlorpyrifos (acute dosing) : *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.5 and 2 mg/kg. *Brain AChE:* NOAEL is 2 mg/kg for both pups and adults. LOAEL is 5 mg/kg for pups and 10 mg/kg for adults. No difference between corn oil and rat milk or diet. Chlorpyrifos oxon (acute dosing): *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.1 and 0.5 mg/kg. *Brain ChE* – not inhibited. Chlorpyrifos (repeat dosing): *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.1 and 0.5 mg/kg/day. *Brain AChE:* NOAEL and LOAEL for both adults and pups are 0.5 and 1 mg/kg/day. Chlorpyrifos oxon (repeat dosing): *RBC ChE:* NOAEL and LOAEL for both adults and pups are 0.01 and 0.5 mg/kg/day. Note although the NOAEL and LOAELs are the same, there are quantitative differences at the LOAEL in some comparisons.
Special Acute inhalation study with ChE inhibition and pharmacokinetic data.	MRID 48139303 (2010) 0, 3.7, 12.9, 22.1 or 53,5 mg/m^3 nominal atmospheric concentrations. Achieved dosage : 0, 0.52, 2.86, 2.21 and 5.7 mg/kg. Acceptable/NonGuideline.	*Plasma and lung ChE:* LOAEL = 3.7 mg/m^3. NOAEL not established. *RBC ChE:* NOAEL = 3.7 mg/m^3. LOAEL = 12.9 mg/m^3. *Brain ChE:* NOAEL = 22.1 mg/m^3. LOAEL = 53.5 mg/m^3. No clinical signs.
870. 7800. 4-week dietary immunotoxicity in rats (females).	48139304 (2010) 0, 0.416, 2.13, 0r 10.7 mg/kg/day. Acceptable/Guideline	No evidence of immunotoxicity at any dose.

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EPA Primary Reviewer: John Doherty, Ph.D., DABT **Signature:** 5/26/11
Risk Assessment Branch V (7509P) **Date:**
EPA Secondary Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV (7509P) **Date:** 5-23-2011

DATA EVALUATION RECORD

TXR NO: 0055409

STUDY TYPE: Non-Guideline Acute and Short Term Repeat Dose Comparative Cholinesterase Study in Rats

DP BARCODE: D380055

P.C.CODE: 059101 (chlorpyrifos) and 065910 (chlorpyrifos oxon)

MRID NO.: 48139301

TEST MATERIAL (Purity): Chlorpyrifos technical (O,O-Diethyl O-(3,5,6-trichloro-2-pyridinyl)ester phosphorothioic acid, Lot # KC28161419. 99.8%) *and* chlorpyrifos oxon (Diethyl 3,5,6-trichloro-2-pyridinyl ester phosphoric acid, lot # 199902031-66, 94.9%).

SYNONYMS: Chlorpyrifos-ethyl for chlorpyrifos, dursban; *and* for the oxon O-analog, Oxydursban, O,O-Diethyl-O-(3,5,6-trichloro-2-pyridinyl)phosphate, Dowco 180

CITATION: M.S. Marty and M.K. Andrus (2010) Comparison of cholinesterase (ChE) inhibition in young adult and preweanling CD rats after acute and repeated chlorpyrifos exposures. Toxicology & Environmental Research and Consulting, The Dow Chemical Company Midland, Michigan 48674, Study ID: 091107. MRID No.: 48139301. Unpublished.
Note: An interim report for this study was submitted under MRID No.: 48070001 (2010). This interim report should not be used for regulatory decisions.

SPONSOR: DOW AgroSciences

EXECUTIVE SUMMARY ***Acute exposure.*** A special *non-guideline* comparative cholinesterase study (2010, MRID No.: 48139301) with CD (Crl:CD(SD) strain rats (8/dose) was conducted to determine the NOAEL and LOAEL for plasma ChE and RBC and brain AChE inhibition following a single oral dose of chlorpyrifos (CPY) and separately for chlorpyrifos oxon (CPO). A *preliminary study* demonstrated the time to peak effect for inhibition of all three enzyme species by CPY in corn oil was 6 hours in pups (at 3 mg/kg) and 8 hours in adults (at 10 mg/kg). Similarly, the times to peak inhibition for CPO in corn oil were determined to be 4 hours for both pups (at 0.5 mg/kg) and adults (at 0.3 mg/kg). The definitive studies consisted of three subparts. In the first subpart, both post natal day (PND) 11 pups and adults rats were dosed by gavage in corn oil. In the second subpart, the PND 11 pups were dosed with CPY in harvested rat milk and the adults were dosed via their diets. In these subparts, CPY doses were 0, 0.05, 0.1, 0.5, 2 or 5 (pups only) or 10 (adults only) mg/kg. In the third subpart, separate

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groups of PND 11 pups and adults were dosed with 0, 0.005, 0.01, 0.05, 0.1, 0.5 or 1 (adults only) with CPO in corn oil.

The study included assessment of body weight and clinical signs. Differences in these parameters were considered at best threshold and to occur when high levels of AChE inhibition were also evident and to be of lesser significance than the comparison of ChE/AChE inhibition and are not discussed in this Executive Summary.

The following table illustrates the NOAEL and LOAELs derived from the acute dosing aspects of this study. Male pups had the same NOAELs and LOAELs as female pups.

Enzyme Source	Acute NOAEL/LOAEL mg/kg (% Inhibition at LOAEL) Pups (male/female %)	Adults (females only)
Plasma ChE:		
CPY – gavage	0.5/2(51%/47%)	0.5/2(54%)
CPY – milk/diet	0.5/2(39%/44%)	0.5/2(58%)
CPO - gavage	0.05/0.1(18%/21% but 51% at 0.5 mg/kg)	0.1/0.5(56%)
RBC AChE:		
CPY – gavage	0.5/2(35% /31%)	0.5/2(19%)
CPY – milk/diet	0.5/2(29%/27%)/	0.5/2(52%)
CPO - gavage	0.1/0.5(46%/47%)	0.1/0.5(36%)
Brain:		
CPY – gavage	2/5(51%/55%)	2/10(57%)
CPY – milk/diet	2/5(42%/56%)	2/10(22%)
CPO - gavage	Not inhibited	Not inhibited

CPY: Comparison of male and female pups. The percent inhibition for male and female pups at the LOAEL was essentially the same for plasma or RBC. When dosed via milk, the male brain was a little less inhibited than females by CPY but the difference (14%) may be assay variation.

CPY: Comparison of pups and adults for inhibition following gavage dosing. The NOAEL for all three enzyme sources for both pups and adults were the same with the brain having a higher NOAEL (2 mg/kg) than for plasma and RBC enzymes (0.5 mg/kg). Quantitative differences at the LOAEL were noted in that the pups (31%) were more inhibited for RBC AChE than adults (19%) at the same dose level. For brain, the pups can be considered more susceptible than adults because although essentially the same degree of inhibition was attained at the LOAEL, the LOAEL is only 5 mg/kg for pups but it is 10 mg/kg for adults.

CPY: Comparison of pups and adults for inhibition following administration via milk to the pups and diet to the adults. Both dosing in milk to pups and via the dietary route to adults resulted in the same NOAEL and LOAELs of 0.5 and 2 mg/kg for plasma and RBC enzymes that was obtained following dosing in corn oil. RBC AChE was more inhibited (52%) in adults than in pups (27%) and the same relationship was apparent for the plasma enzyme. Conversely the brain was more inhibited in female pups (56%) than in adults (only 22%) at the LOAEL even though the LOAELs were 5 mg/kg for pups and 10 mg/kg for adults.

CPO: Comparison of pups and adults for inhibition following gavage dosing. The NOAEL and LOAELs for both plasma and RBC enzymes were established at 0.05 and 0.1 mg/kg. Female

plasma ChE demonstrated an apparent lower NOAEL and LOAEL for pups (0.05 mg/kg and 21% inhibition) than for adults (0.1 mg/kg and 56% inhibition) but at the 0.1 mg/kg dose, the pups were only inhibited 51% suggesting that there is no real difference between pups and adults. RBC AChE demonstrated the same LOAEL where the inhibition was comparable (for both pups (47%) and adults (36%). Brain AChE was not inhibited at any dose by CPO.

Repeat Dosing. In the repeat dosing subpart, PND 11 female pups and adults were dosed with CPY at 0, 0.05, 0.1, 0.5, 1 or 3.5 mg/kg/day for 11 days and plasma ChE and RBC and brain AChE assessments made at 6 hours for pups and 8 hours for adults after the last dose. Similarly, separate groups of PND 11 pups and adults were dosed with 0, 0.01 or 0.5 mg/kg of CPO and assessed 4 fours after the last dose. The study included assessment for clinical sign, body weight and limited FOB and motor activity assessment at the approximate time for peak effect. The following table illustrates the NOAEL and LOAELs derived from the repeat dosing aspects of this study.

Enzyme Source	NOAEL/LOAEL mg/kg (% Inhibition at LOAEL) Pups	Adults
Plasma ChE:		
CPY	0.1/0.5(46%)	0.1/0.5 (46%)
CPO	0.01/0.5 (62%)	0.01/0.5 (76%)
RBC AChE:		
CPY	0.1/0.5 (18%)	0.1/0.5 (20%)
CPO	0.01/0.5 (84%)	0.01/0.5 (87%)
Brain:		
CPY	0.5/1 (19%)	0.5/1 (9%)
CPO	Not inhibited	Not inhibited

CPY: Comparison of pups and adults for inhibition. The same NOAEL and LOAELs resulted for plasma ChE and RBC and brain AChE following repeat dosing. The extent of inhibition at the LOAEL was considered similar at either age.

CPO: Comparison of pups and adults inhibition. Brain enzyme was not inhibited by CPO. The plasma and RBC enzymes demonstrated the same NOAEL and LOAELs of 0.01 and 0.5 mg/kg/day and inhibition at the LOAEL was similar for both enzyme sources.

Analysis of blood for CPY, CPO and TCP. No analytical data for the time to peak effect aspects of this study were presented. The blood samples from the dose response studies were analyzed for CPY, CPO and TCP for only *two* animals of each sex for pups and four adult females. CPO was detected only at higher doses and usually near the level of minimal quantitation. The within group variability of samples often confounded the correlation of blood level of CPY and TCP with inhibition. Therefore, HED reviewers declined from drawing firm conclusions for the blood levels associated with inhibition.

Classification: The classification of this *in vivo* comparative cholinesterase inhibition study is Acceptable/Non-Guideline. The data are considered useful for comparing the pups and adults for *in vivo* inhibition of plasma ChE and RBC and brain AChE. However, the analytical data for CPY and TCP is considered limited in usefulness.

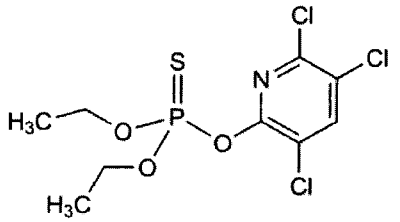
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

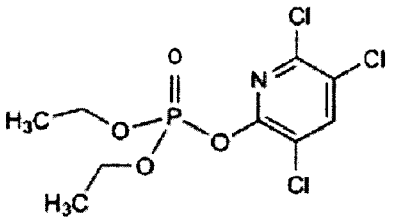
A. MATERIALS:

A1. Test material:

Description: CPY - Chlorpyrifos technical (no physical description provided)
Lot/batch #: (lot# KC28161419).
Purity: 99.8% a.i. (as stated)
CAS # of TGAI: 2921-88-2
Structure:



Description: CPO - Chlorpyrifos oxon (no physical description provided)
Lot/batch #: (lot # 199902031-66)
Purity: 94.9% (as stated)
CAS # of TGAI: 5598-15-2
Structure:



A2. Vehicle and/or positive control: Corn oil – no other description provided.

A3. Test animals:

Species: Rat
Strain: CD rats (Crl:CD(SD))
Age/weight at dosing: 11 days old for pups and ~70 days old for adults at time of dosing.
Source: Charles River Laboratories Inc. (CRL, Portage, Michigan)
Housing: One per cage.
Diet: LabDiet Certified Rodent Chow #5002 (PMI Nutrition). *(ad libitum)*
Water: Municipal *(ad libitum)*
Environmental conditions:
Temperature: 22° ± 1° (maximum ± 3° C
Humidity: 40-70%
Air changes: 12-1-5 times/hour
Photo period: 12 hr light/12 hr dark (on 6 am, off 6 pm)
Acclimation: 7 days.

B. METHODS - STUDY DESIGN:

1. **In life dates:** Start: 9/14/09; End: (6/29/10 – date of final report).

2. **Animal assignment and treatment:** In an acute oral range finding study, adult female rats (3/dose group) were dosed with CPY at 0, 5, 10 or 20 mg/kg or dosed with CPO at 0, 1, 5, or 10 mg/kg in corn oil and sacrificed 5 hours after dosing for assessment of plasma, RBC and brain ChE effects. Subsequently a series of preliminary time to peak effect studies with 4 rats/sex were conducted with CPY and CPO dosed by gavage in corn oil. Additional time to peak effect studies were also done with CPY in harvested rat milk to pups and via the diet to adults. These preliminary studies assessed CPY at 3 mg/kg in pups and 10 mg/kg in adults and CPO at 0.5 mg/kg in pups and 0.3 mg/kg in adults. These preliminary time to peak effect studies established that for the definitive dose response studies the CPY dosed pups should be sacrificed at 6 hour post dosing and the adults at 8 hours post dosing. The CPO dosed animals should be sacrificed 4 hours post posing. For assessing the dose response aspects of this study, animals were assigned to the test groups as indicated in Table 1.

Table 1. Animal assignment and protocol for dosing with chlorpyrifos or chlorpyrifos oxon in the definitive dose response studies.

Part I: Acute Dosing				
Dose (mg/kg)	PND 11-gavage	PND 11- milk	Adult ♀- gavage	Adult ♀- dietary
Chlorpyrifos				
Control	8/sex[1]	8/sex	8	8 (0 mg/kg)
0.05	8/sex	8/sex	8	8 (0.05)[a]
0.1	8/sex	8/sex	8	8 (0.10)
0.5	8/sex	8/sex	8	8 (0.53)
2	8/sex	8/sex	8	8 (2.06)
5	8/sex	8/sex	--	--
10	--	--	8	8 (9.59)
Chlorpyrifos oxon				
Control	8/sex	Not Assessed.	8	Not Assessed.
0.005	8/sex		--	
0.01	8/sex		8	
0.05	8/sex		8	
0.1	8/sex		8	
0.5	8/sex		8	
1	--		8	
Part II: Repeat Dosing (11 days)				
Chlorpyrifos				
	PND 11-gavage		Adults - gavage	
Control	8/sex	Not assessed	8	Not assessed
0.05	8/sex		8	
0.1	8/sex		8	
0.5	8/sex		8	
1	8/sex		8	
3.5	8/sex		8	
Chlorpyrifos Oxon				
Control	8/sex	Not assessed	8	Not assessed
0.01	8/sex		8	
0.5	8/sex		8	

[1] The number of animals listed is based on the number indicated in the results tables.
[a] The number in () is the achieved dose based on food consumption and body weight.

For the studies by gavage, the test materials were administered in a dosing volume of 3 mL/kg of corn oil or milk. The adults were fasted overnight. The pups were kept from their mothers for one hour prior to dosing.

3. **Dose justification.** The dose levels selected for the definitive study were designed to "examine ChE inhibition over the lower portion of the dose response curve. The highest dose levels were selected in induce 50-60% inhibition of brain AChE to anchor the dose-response curve and provide greater certainty in curve fitting at lower doses of these test materials" (ps. 58-59).

4. **Stability, oncentration and homogeneity in milk and diets.** The stability of CPY and CPO in corn oil (study report tables 2 and 3) and CPY in milk (study report table 4) was reportedly demonstrated to show stability up to 12 or 13 days. Homogeneity and concentration in corn oil for CPY (study report table 5) was shown to range from 87.4% to 108.4% and is acceptable. Homogeneity and concentration for CPO in corn oil (study report table 6) ranged from 95.7% to 122.0% with many (17 of 23 assessments) values greater than 100%. Concentration of CPY in milk showed variability ranging from a low of 69% to a high of 93.6% (study report table 7). Overall of the 11 assessments, it appears that only about 80% of the nominal dose was achieved for CPY in the milk. Homogeneity data on CPY in the milk ranged from 30-40% in two instances but was otherwise reasonable. Stability of CPY in the diet has been determined in many studies and should be stable within the 12 hour dietary exposure for this study. Homogeneity and concentration data for the diet preparation (study report table 8) indicated from 85.6% to 101.0% of the target dose concentration and homogeneity was 1.4 and 7.1% for two samplings.

5. **Feed consumption and compound intake (for the dietary exposure to adults).** Feed consumption was assessed during the 12 hour exposure period for the adults in the dietary exposure aspect of the study. CPY intake (as mg/kg) was determined based on feed consumption and body weight. Formula presented on page 66 of study report and the compound intake as dose is presented in Table 1 above.

6. **In-life assessments.**

6a. Body weight. (Reviewer's note: Since this is an acute dosing study, when the rats were sacrificed at the time of peak effect for ChE/AChE inhibition, body weight change over the several hours between dosing and sacrifice has limited value. Body weight assessment was also confounded by fasting the animals prior to dosing.)

6b. Clinical signs. Daily cage side examinations were reported as being made twice daily to look especially for tell-tale signs expected for organophosphate intoxication or other signs (i,e, decreased/increased activity, repetitive behavior, vocalization, in coordination/limping, injury, neuromuscular function – convulsions, fasciculation, tremor or twitches) altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), alterations in fecal consistency and fecal/urinary quantity. For the acute dosing studies, more thorough hand held examination was made at the time of peak effect just prior to sacrifice.

6c. FOB assessments for the repeat dose study.. FOB assessments were reported as being made at the time of peak inhibition and included: hand-held open field observations, rectal temperature, grip performance, body weight and motor activity.

6d. Motor activity for the repeat dose study. Assessments were made at the time of FOB assessments. Adults were tested in an automated system for 8 epochs of 8 minutes duration. PND 20 pups were tested for 6 epochs of 8 minutes duration.

7. Plasma ChE and RBC and brain AChE. The rats were described as being sacrificed at the predetermined times following deep anesthetization by isoflurane/CO_2 inhalation. The thoracic and abdominal cavities were opened for the pups. Blood was collected from the pups be nicking the left ventricle of the heart. For young adult females, blood was collected from the inferior vena cava. Two samples were collected (exact volume not specified), one for plasma ChE and RBC AChE assessment and the other for CPY, CPO and TCP metabolite quantitation. For the analysis of cholinesterase enzymes, the sample was reported to be kept on ice, centrifuged to separate the plasma from the RBCs. The RBCs were diluted 1:20 with 1% Triton X-100. Both plasma and lysed RBCs were frozen at -80°C and shipped to the WIL Laboratory for analysis of ChE and AChE. The protocol or SOP (effective date September 12, 2006) that the WIL Laboratories used for assessment of ChE and AChE was provided to HED upon request from the reviewer.

Brain AChE was assessed following removal of the brain from pups and adults, rinsing in saline, sectioning into right and left hemispheres and the right hemisphere weighted. The sections were quick frozen in liquid nitrogen and sent to the WIL Laboratory for further preparation and analysis of AChE.

8. Analysis of Blood for CPY, CPO and TCP. There was no description of the methods used to assess for CPY, CPO and TCP or a description of storing the sample prior to assessment. Only references to papers by Brzak et al (1998, J. Anal. Toxicol. **22**:203-210) and Mattsson et al (2000, Toxicol. Sci. **53**:438-446) were provided.

9. Statistical Assessments. Statistically significant differences indicative of inhibition of ChE/AChE were assessed for by a two-sample t-test or a one way analysis of variance (ANOVA) using dose as a factor. Descriptive statistics (mean and standard deviation) were reported to be used for blood levels of CPY, CPO and TCP.

The study report also described in considerable detail the statistical analysis of clinical signs, body weight data, FOB including body temperature and motor activity. However, since these endpoints were not affected (or at best considered threshold for an effect) and since the main aspect of this study is to determine the NOAEL and LOAEL for ChE/AChE inhibition, the extensive statistical assessments for these parameters will not be repeated here. Please refer to the study report if needed.

II. RESULTS

A. ACUTE AND REPEAT DOSING COMMON RESULTS

1. Clinical signs, FOB (limited), Body Weight and Food Consumption Effects for Both Acute and Repeat Dosing Studies with CPY and CPO for PND 11 Pups and Young Adults.

Clinical signs, FOB and motor activity: The following is the summary of the assertion in the study report with regard to the results of the clinical signs, FOB and motor activity assessments.

Pilot time to peak effect studies (p. 71-72 – *No treatment related effects (assessment for clinical signs only) with either CPY or CPO.*
Acute Dose response studies (p. 72-73) - *No treatment related effects in either pups or adults (assessment for clinical signs only) with either CPY or CPO.*
Repeated Dose response studies (p. 73- 82) – *With the exception of certain isolated incidents possibly, but not definitely resulting from treatment, there were no consistent effects of treatment with either CPY or CPO.*

Body weight and food consumption: Adult females exposed by the dietary route had either no gain or lost weight at the 10 and 20 mg/kg dose groups in the time to peak effect study and a somewhat similar decrease was noted in the definitive study at the10 mg/kg dose group. It is noted that dosing was done following over night fasting and there was a noticeable fasting effect on body weight. The limited data presented did not indicate any definite treatment related effects in the repeated dose experiments with either CPY or CPO in PND 20 pups or adult females.

In summary. HED reviewers inspected the many data tables (Tables 9 to 98) in the study report and concluded that there were some differences in the highest dosed groups but generally these seem to occur in only a few animals. Response to stimuli in pups appears to be an example. Due to the low frequency, HED concludes that the high doses of CPY and CPO would be a minimal threshold effect that would need to be verified by testing at even higher doses. HED considers that the main aspect of this comparative ChE study is to determine the NOAEL and LOAELs for ChE/AChE inhibition and that whatever effects on clinical signs, FOB or body weight would occur at doses at or higher than the LOAEL for inhibition of at least the blood enzymes. HED does not consider at this time that further review for detecting possible subtle differences in these parameters that might occur at higher doses would result in advancing the knowledge of the effects of CPY or CPO.

2. Plasma ChE and RBC and Brain AChE Values and Variance in Untreated Animals.

In order to assess the reliability of the ChE/AChE data in this study, Table 2 was prepared to show the mean and standard deviation and variance (standard deviation as a percent of the mean) for both 11 day old male and female pups and for adult females for both the control groups for the chlorpyrifos and chlorpyrifos oxon studies.

Expected Values/Accuracy. Table 2 shows that for all six pup control groups the mean value for either plasma ChE or RBC or brain AChE were similar for a given enzyme species. There was no *marked* difference between males and females. For pups, the plasma ChE had the lowest

specific activity, RBC AChE was about 4.5 times higher than plasma and the brain had the highest specific which was about 3.7 times higher than RBC AChE. For adults, the RBC AChE was about 2.6 times higher than plasma and brain was about 9.6 times higher than RBC AChE. Plasma ChE and brain AChE were higher in adults than in pups but RBC AChE was similar in both pups and adults. Plasma and brain activities were higher in adults than in pups whereas RBC activity was less in adults than in pups. The values for plasma ChE, RBC and brain AChE are considered reasonably comparable with respect to published values as well as other studies in HED files.

Precision. The plasma ChE assessment in adult females had the poorest precision (22.5 to 37.5%). The precision for all other groups ranged from <4% (adult brain) to 23.2% (pup RBC). Overall, the precision is considered typical for a ChE/AChE assessment.

Table 2. Variance [&] in control groups for plasma ChE and RBC and brain AChE. (U/L)

Enzyme Source		11 Day Old	Adults
Plasma ChE	Males - CPY	1407.4±111.2 (7.9%)	--
	" CPO	1444.9±118.3 (8.2%)	--
	Females- CPY	1421.0±106.1 (7.5%)	2142.6±802.5 (37.5%)
	" CPO	1487.4±209.7 (14.1%)	2105.9±562.2 (26.7%)
	Males –CPY (a)	1612.9±184.5 (11.4%)	--
	Females-CPO(a)	1586.4±137.4 (8.7%)	1753.8±394.9 (22.5%)
RBC AChE	Males - CPY	6891.0±782.3 (11.4%)	--
	" CPO	6564.3±512.71 (7.8%)	--
	Females- CPY	6431.5±776 (12.1%)	5409.3±350.5 (6.5%)
	" CPO	6287.3±856.3 (13.6%)	5630.3±906.2 (16.1%)
	Males –CPY (a)	6304.5±1459.8 (23.2%)	--
	Females- CPO(a)	6324±648.1 (10.3%)	5337.0±304.8 (5.7%)
Brain AChE	Males - CPY	22764.8±3335.7 (14.7%)	--
	" CPO	25538.5±1711.21 (6.7%)	--
	Females- CPY	24628.8±3768.1 (14.3%)	54727.8±1283.2 (2.3%)
	" CPO	22993.6±4167.9 (18.1%)	52826.4±2036.4 (3.9%)
	Males – CPO (a)	23910.8±1872.8 (7.8%)	--
	Females- CPY(a)	26410.1±3026.3 (11.5%)	53887.6±1241.7(2.3%)

(a) – Pups were dosed via milk and adult females were dosed dietary route. -- No data.
Sources: Male pups CPY – Table 117(p.330); Male pups CPO – Table 122 (p. 335); Female pups CPY – Table 118 (p.331); Female pups CPO Table 123 (p. 336); Male pups – milk – Table 127 (p. 340); Female pups – milk – Table 128 (p. 341); Adult females CPY – Table 131 (p.344); Adult females CPO – Table 134 (p.347); Adult females – diet – CPY – Table 137 (p.350).

[&] Variance as calculated by the reviewer.

Variability of controls over time. Table 3 demonstrates the variability of plasma ChE and RBC and brain AChE over time for the three time to peak effect studies where there was a control group assessed at six (up to 24 hours) or seven (up to 48 hours) time points following dosing up to 48 hours. The table shows that the standard deviation for the means of these times for RBC and brain AChE is less than 10% when expressed as a coefficient of variation. RBC AChE has more variability than brain AChE based on the standard deviation. However, outliers as determined by the difference between the high and low value can vary when expressed as a percentage of the mean by as much as 31% as indicated by the brain data for the CPY controls in the milk dosing study. The overall point of this comparison is that the variability is what might be expected for time course within the time frame of up to 48 hours. Outliers occur and their presence needs to be considered in the overall evaluation of the study.

Table 3. Variability over time for plasma ChE, RBC and Brain AChE in females.			
Group	Plasma ChE	RBC AChE	Brain AChE
CPY – pups in corn oil Table 104 (p.317)	1307-1567 (260)	5897-7142 (1245 or 19%) 6666±388 (5.8%)	26942 – 33294 (6352 or 22%) 28762±2257 (7.9%)
CPY- – pups in milk Table 108 (p321)	1240-1512 (272)	5459-6926(1467 or 23%) 6330±523 (8.3%)	22024-30043(8019 or 31%) 25581±303 (1.2%)
CPY– adults in corn oil Table 110 (p323)	1824-3030(1206)	4249-5578(1329 or 26%) 5102±507 (9.9%)	50152-53899(3747 or 7.2%) 52085±1243 (2.4%)

Data for the RBC and brain are top line - the high and low values (the difference and difference as % of the mean; lower line, the mean and standard deviation (variance). Mean and variance data were calculated by the reviewer but were not calculated for plasma ChE.

B. CPY – ACUTE DOSING RESULTS

1. **Test Chemical Analytical Data (Study report tables 2 to 8). Refer to Section IB.4 above.**

2. **Clinical signs.** The conditions of several moribund animals were described and some of these were in control groups. Since assessment of ChE/AChE inhibition is the main focus of this study, and because assessment of the FOB parameters (see below) corroborates the clinical signs, further analysis of the clinical signs was not considered warranted by the HED reviewers.

3. **FOB assessments.** The study report asserts that there were no alterations attributed to treatment in the FOB assessments for body weight, rectal temperature, grip performance, or motor activity for any of the groups dosed with either CPY or CPO.

3. Body Weight. Body weight effects for this type of study may be expected to vary because of fasting. Since the animals in the dose response aspects of the study were sacrificed at the time to peak effect that was only several hours postdosing, it is difficult to interpret minor alterations in body weights differences thus body weight data are not considered a critical endpoint for this acute study.

4. Food Consumption

Same comments as above for body weight effects.

5. Plasma ChE and RBC and Brain AChE.

5a. *Time to Peak inhibition.* In a preliminary study, the time to peak inhibition following a single oral dose of chlorpyrifos by gavage was established as shown in Table 4. From these data it was determined that the time to peak effect for pups is 6 hours and the time to peak effect for adults is 8 hours. It is noted that both pups and adults had reversal of ChE or AChE inhibition after their time to peak effect and by the 24 hour assessment time. The study report compares the time to peak effect obtained from this study with times to peak effect obtained from studies reported in the literature (Study Report Text Table 1, page 50 for pups and Text Table 2, page 54 for adult females) and although there is some variation in response time, the selections made for time to peak effect for the definitive study are considered reasonable by the HED reviewers. The Study

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Report (SR) also presents many figures attempting to demonstrate that the data conducted to determine the times to peak effect in both pups and adults are reasonably consistent with the times predicted by modeling (Text Figures 31-34, 39-43 and 48-50). HED declines from commenting on the relationship between these data and the proposed models.

Table 4. Time to peak inhibition following gavage dosing with CPY to female pups and adults.

Hour	Pups (3 mg/kg) SR Tables 103 and 104			Adults (10 mg/kg) SR Tables 109 and 110		
	Plasma	RBC	Brain	Plasma	RBC	Brain
0	1463±43	6615.5±517.8	27390.3±1814.6	2433.3±592.7	5380±316.5	50152.3±2778.6
2	44%	20%	19%	73%	39%	None
4	72%	61%	39%	79%	71%	31%
6	**70%**	**72%**	**38%**	77%	81%	36%
8	66%	70%	33%	**92%**	**92%**	**60%**
12	72%	74%	37%	88%	90%	55%
24	56%	60%	28%	64%	76%	41%

Data for the dosed groups are percent inhibition. There were generally 4 animals per assessment time.
Note: The gray bar shows the inhibition at the time to peak effect.

The times to peak effect following administration of chlorpyrifos to pups in milk and to the adults via the dietary route were also determined and the results reported are shown in Table 5. It is noted that the true time to peak effect in adults is not really established by the data provided.

Table 5. Time to peak inhibition following milk dosing in pups and dietary (12 hours) dosing with CPY to female pups and adults.

Hour (Post Dosing)	Pups (3 mg/kg) Table 108, p. 321			Adults (10 mg/kg-nominal dose) Table 114, p. 327.(a)		
	Plasma	RBC	Brain	Plasma	RBC	Brain
0	nd	nd	nd	Not reported	5659.2±1661.7	55305.6±3366.9
2	802±231.4*	4027±955.2*	19975.3±3683.5	"	175.6±191.8	
4	735.3±122.5*	3812.3±1160*	20572.8±3076.8	"	39.6±38.2	
6	761.7±122.2*	4460±1050*+	23263.7±2370.5			
8	462.5±125.7*	1871.3±1015*	15483.3±3410*	"	175.2±108.4	43257.6±4968.8
12	793.3±187.1*	4217±1041*+	22411±1268.6*			
24	540.8±87.6*	2424.7±861.2*	22307.3±2891*			
48	677±135.5*	2771±745.2*	23407.3±3193*			

There were 6 animals per assessment time for pups and 5 for adults. nd = not reported.
(a). The adults did not have 0 time values and the control group value is entered above. Also no 2 or 4 hour values were provided for the brain enzyme.
* Significantly different from the control at $p < 0.05$ using Dunnett's test. + signifies an increase in activity.

5b. Dose *response and determination of the NOAEL and LOAEL following gavage administration of chlorpyrifos.*

Table 6 demonstrates the dose response for inhibition at the time to peak effect for both pups and adults. The NOAEL and LOAEL as determined by the HED reviewer are also presented in the table. The HED reviewer and study report author are in agreement with regard to the NOAEL and LOAEL for both pups and adults.

Table 6. Comparison of inhibition of plasma ChE and RBC and brain AChE in pups and adults following an acute dose of chlorpyrifos by gavage in corn oil.

Dose Mg/kg		Pups SR Table 117, p. 330 ♂, 118, p. 331♀			Adults SR Table 131, p. 344		
		Plasma	RBC	Brain	Plasma	RBC	Brain
Control	♂	1407.4±111.2	6891.0±782.3	22764.8±3335.7	--	--	--
	♀	1421±106.1	6431.5±776	24628.8±3768.1	2142.6±802.5	5409.3±350.5	54727.8±1283.2
0.05	♂	1440.3±221.1	7034±923.3	25891±2664.1	--	--	--
	♀	1408.6±150.1	6673.5±633.6	25124.8±2986.8	2317.3±634.9	5146±533.3	54461.4±2300.7
0.1	♂	1494±177	7194.8±860.7	24338.9±1508.2	--	--	--
	♀	1373.9±200.3	6518±677	24910.4±3080.2	1943.8±683.8 9%	5494±356.9 S	51115.9±2484 (7%)
0.5	♂	1300.6±89.1 (8%)	6537.5±544.3 (5%)	25201.8±1846.7 (+)	--	--	--
	♀	1283.4±136 10%	6345±1307.6	25372.4±2069.2 (+)	1873.9±262.3 12% (T)	5974.5±1150.1 (+)	52242.5±1585.6 (4%)
2	♂	689.5±63.3 51%*	4433.5±590.1 35%*	22345.5±2909.6 7% (T)	--	--	--
	♀	752.4±140.8 47%*	4440.8±392.2 31%*	22932.9±3188.3 7%(T)	980.6±283.6 54%*	4359.8±536.2 19%*	52193.5±2579.5 (4%)
5	♂	320.8±54.1 77%*	804.3±248.8 88%*	11162.9±2820.5 51%*	--	--	--
	♀	313±68.8 78%*	873.3±281.5 86%*	10955.4±1516.9 55%*	--	--	--
10	♀	--	--	--	283.1±79.6 87%*	851.3±622.2 84%*	23276.3±11567.4 57%*
NOAEL		**0.5 (T)**	**0.5**	**2**	**0.5 (T)**	**0.5**	**2**
LOAEL		**2**	**2**	**5**	**2**	**2**	**10**

(T) Threshold – 12 % or less inhibition of plasma ChE is not considered to be toxicologically relevant. It is recognized that some may disagree and set the NOAEL lower but this will not change the overall conclusion that neonates and adults are not really different. + indicates an increase in activity.
Sources: Male pups – Table 117 (p.330), female pups – Table 118 (p.331); adult females – Table 131 (p.344).
There were 8 animals per group.
*Statistically different from the control at 0.05 using Dunnett's test.

5c. *Dose response and determination of the NOAEL and LOAEL following dosing in milk to pups and dietary dosing with chlorpyrifos.*

Table 7 demonstrates the dose response for inhibition at the time to peak effect for plasma ChE and RBC and brain AChE following dosing via milk in pups and by the dietary route in adults. These NOAELs and LOAELs are the same as in Table 5 following dosing via gavage in corn oil. Quantitatively, or extent of inhibition at the same dose are very similar for pups but there are some differences for the adults dosed via the dietary route as compared with dosing via gavage. In particular, at the LOAEL, gavage dosing results in only 19% inhibition for RBC enzyme but 52% for dietary dosing. At the next higher dose, both administration methods result in similar near total inhibition. In contrast, brain inhibition at the LOAEL is lower (22%) following dietary administration than by gavage administration (57%).

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Table 7. Comparison of inhibition of plasma ChE and RBC and brain AChE in pups and adults following and acute dose of chlorpyrifos via milk versus in pups versus dietary exposure in adults.

Dose Mg/kg		Pups (via milk)			Adults (via diet)		
		Plasma	RBC	Brain	Plasma	RBC	Brain
Control	♂	1612.9±184.5	6304.5±1459.8	23910.8±1872.8	--	--	--
	♀	1586.4±137.4	6324±648.1	26410.1±3026.3	1753.8±394.9	5337±304.8	53887.6±1241.7
0.05	♂	1629.1±180.7	6016.5±1384.9	24489.1±3432	--	--	--
	♀	1645.5±170.8	6196.5±613.7	26113.3±2978.7	1372.6±249 (22%???)	5164±575 (3%)	52699.8±2265.1 (2%)
0.1	♂	1572,9±238.8	6590.8±923.9	25278.8±2155.3	--	--	--
	♀	1475.8±179.3 (7)	6500.8±978.9 (+)	26608.9±2104.8	2057.5±457.5 (+)	5379.5±524.6 (+)	53020.3±1697.8 (2%)
0.5	♂	1510.4±142.4 (11%)	6320.3±1027.5 (+)	25324.6±2815.4 (+)	--	--	--
	♀	1410±160.2 (11)	5876.8±1504.7 (7)	26969.4±1413	1648±364.3 (6%)	5218.8±575.3 (2%)	53844.4±1736.7 (Same)
2	♂	985±198.3* (38.9)	4458.5±1915.7 (29%)	24760.1±2743.6 (+)	--	--	--
	♀	895.8±219.9 (44)*	4599.8±1758.9 (27)*	22201±4811.4 (16 Threshold?)	736.9±190.1 58%*	2547.8±445.8 52%*	51293.6±1639.1 (5%)
5	♂	471.6±507.5* (71)	1805±1706.2 (71%)	13918.4±4866.7 (42%)*	--	--	--
	♀	340.9±238.8 (79)*	1374.8±1633.5 (78)*	11511.3±5332 (56)*	--	--	--
10	♀	--	--	--	229.5±82 87%*	167±146 97%*	41125.4±7649.4 24%*
NOAEL		**0.5**	**0.5**	**2(Threshold?)**	**0.5**	**0.5**	**2**
LOAEL		**2**	**2**	**5**	**2**	**2**	**10**

*Significantly different from control at 0.05 using Dunnett's test. Based on 8 rats per dose. + indicates an increase in activity.
Sources: Male pups – table 127 (p.340); Female pups – Table 128 (p.341); Adult Females – Table 137 (p.350).
There were 8 animals per group.
*Statistically significantly different from the control at 0.05 by Dunnett's test.

C: CPO ACUTE DOSING.

1. Plasma ChE and RBC and Brain AChE

1a. *Time to peak inhibition.* In a preliminary study, the time to peak inhibition following a single oral dose of chlorpyrifos was established as shown in Table 8. Brain AChE was not inhibited in either pups or adults. From these data it was determined that the time to peak effect for pups is **4 hours** for both plasma and RBC enzymes. The RBC AChE response was not robust at a dose of 0.3 mg/kg but the laboratory selected the time to peak effect for adults at **4 hours** and provided considerable discussion including the predicted time to peak effect based on models (SR pages 91-93). The four hour time interval for time to peak effect for RBC enzyme is consistent with maximal inhibition of plasma ChE at 4 hours. The 52%, 21% and 56% inhibition for adult plasma ChE for 4, 6 and 8 hours is not explained. The 22% apparent lower activity for RBC AChE at 24 hours is not considered actual inhibition. Again it is noted that there is apparent reversal of inhibition for plasma ChE following the peak time for inhibition. Overall, HED reviewers consider selection of 4 hours as the time to peak effect a reasonable choice.

Table 8. Time to peak inhibition following gavage dosing with chlorpyrifos oxon in corn oil to pups and adults.

Hour	Pups (0.5 mg/kg) SR Tables 105 and 106			Adults (0.3 mg/kg) SR Tables 111 and 112		
	Plasma	RBC	Brain	Plasma	RBC	Brain
O	1463±43.4	6615.5±517.8	27390.3±1814.6	2433.3±592.7	5380±316.5	50152.3±2778.6
2	56%	55%	None	50%	9%	(+6%)
4	**60%**	**71%**	None	**52%**	14%	(5%)
6	52%	68%	None	21%	12%	None
8	47%	62%	None	**56%**	**3%**	None
12	40%	51%	None	24%	10%	(+7%)
24	19%	41%	None	(+13%)	22%	None

+ reading is higher than control. There were 4 animals per group.

1b. *Dose response and determination of the NOAEL and LOAEL following gavage administration of CPO.* Table 9 demonstrates the dose response to CPO following gavage administration in corn oil.

Brain AChE was not shown to be inhibited even at the highest dose when RBC AChE was inhibited to 71% and this is consistent with the time to peak effect study (see Table 8 of this DER). The rat pups were apparently more sensitive to inhibition of plasma ChE than to RBC based on the inhibition in both sexes at 0.1 mg/kg (Table 9).

Table 9. Comparison of inhibition of plasma ChE and RBC and brain AChE in pups and adults following and acute dose of CPO by gavage in corn oil.

Mg/kg	Pups SR Table 122 (♂) and 123 (♀)			Adults SR Table 134		
	Plasma	RBC	Brain	Plasma	RBC	Brain
Control ♂	1444.9±118.3	6564.3±512.7	25538.5±1711.2	--	--	--
♀	1487.4±209.7	6287.3±856.3	22993.6±4167.9	2105.9±562.2	5630.3±906.2	52826.4±2036.4
0.005 ♂	1361.8±115.08 6%	6174.3±827.9 6%	24558±2114 4%	--	--	--
♀	1428.4±124.8 16%	6145.8±1556.6 2%	22435.6±1790 (3%)	--	--	--
0.01 ♂	1465.6±127.1 ~	5564.5±1280.8 15%	23708.6±2579.8 (7%)	--	--	--
♀	1433.1±172.2 ~~	6227.3±1233.8 ~	22357.9±2619.6 3%	1921.9±368.9 9%	5509.8±661.8 2%	52517.1±1502.5 ~
0.05 ♂	1378.3±209.8 5%	6158.5±807.3 6%	24800±3115.5 3%	--	--	--
♀	1351.5±200.2 9%	5444±1025.2 12%	24178.3±1236.0 +	1983.6±374.1 6%	5829.8±401.5 +	53202.8±2367.3 +
0.1 ♂	1182.3±189.6 18%	5506.3±976.7 16%(b)	24798.4±2811.5 3%	--	--	--
♀	1179±123.8* 21%	5651.3±1274.3 10% (b)	22201.6±3626.5 3%	2088.4±736.2 ~	5654.5±209.7 ~	51750.6±991.6 2%
0.5 ♂	738.1±72.2* 49%	3534±488.7* 46%	23489.4±3132.9 (8%)	--	--	--
♀	722.8±61.5* 51%	3328.5±771.8 47%	22462.1±2964.3 ~	930.6±369.2 56%*	3572±905.3 36%*	51168.5±1299 3%
1 ♀	--	--	--	502±140.6 76%*	1337.5±497.2 76%*	51045.6±1829.6 3%
NOAEL **LOAEL**	**0.05** **0.1**	**0.1** **0.5**	**Not inhibited.**	**0.1** **0.5**	**0.1** **0.5**	**Not inhibited**

*Significantly different from control at 0.05 using Dunnett's test. Based on 8 rats per dose group.

(T) Threshold – 12 % or less inhibition of plasma ChE is not considered to be toxicologically relevant. It is recognized that some may disagree and set the NOAEL lower but this will not change the overall conclusion that neonates and adults are not really different.
(b) There is no dose response and both males and females show similarly lower activity at the lower dose.

D.Comparison of CPY and CPO following acute dosing.

Table 10 demonstrates that except for brain AChE which was not inhibited by CPO, the NOAEL and LOAELs are much lower for CPO when compared to CPY and this would be expected.

Table 10. Comparison of the NOAELs and LOAELs in mg/kg for both CPY and CPO for females following gavage administration in corn oil[1].

		NOAEL/LOAEL (% inhibition at LOAEL) Pups	Adults
Plasma ChE	CPY	0.5/2(47%)	0.5/2(54%)
	CPO	0.05/0.1(21%)	0.1/0.5(56%)
Factors[2]		10/20	5/4
RBC AChE	CPY	0.5/2(31%)	0.5/2(19%)
	CPO	0.1/0.5(47%)	0.1/0.5(36%)
Factors		5/4	5/4
Brain AChE	CPY	2/5(55%)	2/10(57%)
	CPO	Not inhibited	Not inhibited

[1] From tables 6 and 9 of this DER. [2] The relative difference between the NOAEL/LOAEL as NOAEL for CPY/NOAEL for CPO and LOAEL for CPY/LOAEL for CPO.

E. CPY REPEAT DOSING RESULTS

The times to peak effect were the same as for the acute study, 6 hours for pups and 8 hours for adults following the last dose. Table 10 demonstrates the dose response for inhibition of plasma ChE and RBC and brain AChE in both pups and adults following 11 days dosing in corn oil. Qualitatively, the NOAELs and LOAELs are lower for repeat dosing than for acute dosing as would be expected. Repeat dosing results in the same NOAELs and LOAELs for both pups and adults. It is noted, however, that the 0.1 mg/kg/day dose level resulted in an apparent 16% inhibition of RBC AChE for adult females. HED reviewers consider the 0.1 mg/kg/day dose level is at best a threshold for RBC inhibition in adult females since there was only an increase to 20% apparent inhibition at the next dose of 0.5 mg/kg/day which is 5 times greater. Also, plasma ChE is more inhibited at the 0.5 mg/kg/day dose than RBC AChE casting additional doubt that the 16% decrease in activity is actually inhibition. Quantitatively (or extent of inhibition at the same dose), the pups are *not* much more sensitive than adults. For example, female brain AChE is inhibited 19% for pups and 9% for adults at 1 mg/kg but at 3.5 mg/kg, pups are inhibited 59% and adults 69%. The 10% difference at 1 mg/kg is probably within experimental variability rather than a definite CPY effect. The study report states (p. 114) that the NOAEL for ChE inhibition "across all tissues was 0.1 mg/kg/day with repeated daily exposures from PND 11-21." The study report also states that the 6% apparent lower activity for the brain enzyme in the 0.5 mg/kg/day dose group is not inhibition.

Table 11. Comparison of inhibition of plasma ChE and RBC and brain AChE in pups and adults following repeat dosing with CPY via gavage in corn oil.

Dose Mg/kg	Pups (♂ Table 141, ♀ Table 142, pages 354-355)			Adults (Table 150 p. 363)		
	Plasma	RBC	Brain	Plasma	RBC	Brain
Control ♂	905.3±111.3	6410±1605	43264.5±1500.4	--	--	--
♀	928.4±122.4	5951±2082.0	42289.4±1615.9	2253.9±744	4903.3±275.8	51977.8±1802.6
0.05 ♂	949.3±148	5516.5±1179.7	43558.4±1981.1	--	--	--
♀	921.1±45	6388±1021*+	44725.8±2598	2892.3±1051.8	4654.9±712.7	51929±1031
0.1 ♂	901.9±135.8	5449.8±1293.6	42656.8±1575.7	--	--	--
♀	864.1±95.3	5882±1281.5	43498.6±1409	2616.6±770.4	4119.3±488.7* 16% (T)	51990±3290.9
0.5 ♂	645±58,2* 29%	4054±111.8* 37%	40779.8±1864.7 6%	--	--	--
♀	713.3±172.7* 23%	4870±829 18% ns	42809.6±1257.7 ~	1220.3±561.8* 46%	3946±722.4* 20%	51694±1861.8 ~
1 ♂	507.6±69.7* 44%	2478±532.5* 61%	31005.9±4154.3* 28%	--	--	--
♀	519±64* 44%	3333±821* 44%	34265.8±2009* 19%	691±316* 69%	1334.8±395.1* 73%	47357.3±1937.9* 9%
3.5 ♂	186.6±22.2* 79%	539.8±332.2* 92%	13898±987.9* 68%	--	--	--
♀	266.3±51.3* 71%	723±264.2* 88%	17344.1±2414* 59%	262.4±73.4 88%	134.5±184.9* 97%	16092.6±2361.7* 69%
NOAEL LOAEL HED	**0.1 0.5 (23%)**	**0.1 0.5 (18%)**	**0.5 1 (19%)**	**0.1 0.5 46%**	**0.1(16%) -T 0.5 (20%)**	**0.5 1 (9%)**

*Significantly different from control at 0.05 using Dunnett's test. Based on 8 animals/group.
T= considered a threshold dose.

F. CPO REPEAT DOSING RESULTS

Table 12 demonstrates the dose response for inhibition by CPO following repeat dosing and assessments made 4 hours after the last dose. The data establish NOAEL and LOAELs of 0.01 and 0.5 for both plasma ChE and RBC AChE and similar to the acute study, there was no inhibition of brain AChE.

Table 12. Comparison of inhibition of plasma ChE and RBC and brain AChE in female pups and adults following repeat dosing with CPO via gavage in corn oil. Based on 8 animals/group.

Dose Mg/kg	Pups (Table 146, p. 359 ♂, 147, p 360 ♀)			Adults (Table 153, page 366)		
	Plasma	RBC	Brain	Plasma	RBC	Brain
Control ♂	905.3±111.3	6410±1605	43264.5±1500.4	2253.9±744.2	4903.3±275.8	51977.8±1802.6
♀	928.4±122.4	5951±2082	42289.4±1615.9			
0.01 ♂	933.4±135.5	5848.5±1152.8 (9%)	43428.9±1394.6			
♀	902.6±94.6	5163.3±1198.6	44273.3±1639	2813.5±1122.1 +25%	5226.8±1065.2 +7%	51480.3±1954
0.5 ♂	344.4±50.8* (62%)*	1012.6±536.8 (84%)*	43003.1±1747.3			
♀	361.9±33.5 (61%)	813.3±304.4 (86%)	38762.3±12286 8% ns	540.5±160 76%*	619.3±227.6 87%*	51160.6±2203.6
NOAEL LOAEL	**0.01 0.5 (62%)**	**0.01 0.5 (84%)**	**Not inhibited**	**0.01 0.5 (76%)**	**0.01 0.5 (87%)**	**Not inhibited**

*Significantly different from control at 0.05 using Dunnett's test. Based on 8 animals/group.

G. PHARMACOKINETIC DATA

There are at least 10 tables of analytical data for CPY, CPO and principal metabolite TCP in blood in the study report. These are for:

-Single dose CPY in corn oil to pups (119); m/f* -2 animals/group/sex
-Single dose CPO in corn oil to pups (124); m/f ibid
-Single dose CPY in milk to pups (129); m/f ibid
-Single dose CPY in corn oil to adults (132); f -4 animals/group
-Single dose CPO in corn oil to adults (135); f ibid
-Single dose CPY in diet (12 hours) to adults (138); f ibid

-Repeat dose CPY in corn oil to pups (143); m/f -2 animals/group/sex
-Repeat dose CPO in corn oil to pups (148); m/f ibid
-Repeat dose CPY in corn oil to adults (151); f - 4animals/group
-Repeat dose CPO in corn oil to adults (154); f ibid

*Table numbers are in (). m/f = male and female data are in the same table. f = date for adult females only.

The analytical data with CPO is considered to be of limited value because CPO was only detected at the higher doses (i.e. 5 mg/kg dose) and at low levels near the limit of quantitation.

For the pups, there were data from only two individuals from each sex when there were 8 to 10 per sex when both sexes were dosed were available. When only adult females were dosed, there were data from 4 females of 8 available animals.

Comparison of blood level of CPY and TCP with RBC inhibition in pups and adults following dosing with CPY. Table 13 shows the extent of inhibition for RBC AChE at the LOAEL and also for some cases at higher doses where there was increased inhibition together with the two values for pups and the four values for adults for CPY and TCP. The individual values are presented in this table rather than their means to show the variability. HED reviewers have drawn the following conclusions from these data.

-In many cases there is variability in the results meaning that for the m/f sets where only two animals/sex were assessed the data are not considered to be reliable.
-When four adult females are assessed the mean may be more reliable but there is still considerable variability.
-Attempts to estimate the blood level of either CPY or TCP with inhibition and comparison between pups and adults is considered by HED reviewers to be confounded because of the variability in the precision of the analytical data. This may result in part because only 2 pups and 4 adults were assessed when 8-10 were available.

Table 13. Comparison of the RBC AChE inhibition at the LOAEL and above for pups and adults with CPY and TCP in blood.

Dosing conditions		Pups		Adults	
		Chlorpyrifos			
		Inhibition	Blood Level	Inhibition	Blood Level
Single dose in corn oil	M	35%	(15.5, 10.3)		--
LOAEL = 2	F	31%	(4.8, 12.7)	19%	(1.29, 1.15, 1.11, 0.866)
5 mg/k /10 mg/kg	M	88%	(234, 37.5)		--
	F	86%	(43, 39.4)	84%	(69.8, 35.3, 15.8, 8.92)
			p.332		p.345
Single dose in milk or	M	29%	(6.53, 16.1)		--
dietary LOAEL = 2	F	27%	(2.34, 7.84)	52%	(<LOQ to 0.131)
5 mg/kg/10 mg/kg	M	71%	(25.9, 18.2)		--
	F	78%	(40.4, 129)	97%	(0.747, 4.69, 3.24, 2.10)
			p.342		p.351
Repeat dose in corn oil	M	37%	(0.582, 0.853)		--
LOAEL = 0.5	F	18%	(0.369, 0.582)	20%	(<LOQ to 0.189)
1 mg/kg	M	61%	(1.76, 1.82)		--
	F	44%	(1.95, 1.23)	73%	(0.402, 0.464, 0.237, 1.07)
3.5 mg/kg	M	92%	(14.4, 7.27)		--
	F	88%	(7.79, 5.86)	97%	(1.65, 2.33, 2.47, 2.37)
			p.356		p.364
			TCP		
Single dose in corn oil	M	35%	(988, 1115)		--
LOAEL = 2	F	31%	(831, 1467)	19%	(476, 422, 651, 428)
5/10 mg/kg[(a)]	M	88%	(1298, 1807)		--
	F	86%	(2439, 1973)	84%	(3325, 2789, 3276, 1824)
			p.332		p.345
Single dose in milk or	M	29%	(922, 945)		--
dietary LOAEL = 2	F	27%	(786, 1491)	52%	(222, 230, 231, 186)
5/10 mg/kg	M	71%	(1739, 1870)		--
	F	78%	(1791, 1506)	97%	(1063, 1796, 983, 906)
			p.342		p.351
Repeat dose in corn oil	M	37%	(80.2, 145)		--
LOAEL = 0.5	F	18%	(103,175)	20%	(67.3, 191, 152, 374))
1 mg/kg	M	61%	(373, 178)		--
	F	44%	(149, 307)	73%	(392. 304,271, 440)
3.5 mg/kg	M	92%	(827, 589)		--
	F	88%	(1196, 1191)	97%	(1202, 2341, 1739, 1970)
			p.356		p.364

Data for chlorpyrifos or TCP are ng/gm.
– Not assessed.

Comparison of blood level of CPY and TCP with brain AChE inhibition following dosing with CPY. Table 14 shows a comparison of brain AChE inhibition at the LOAEL for pups and adults with chlorpyrifos and TCP in blood. In general the CPY and TCP levels increase with administered dose, which would be expected but neither of these are the active form of the inhibitor (CPO is). TCP levels are the highest but also show variability such as more than 2 fold for a given time and dose set.

Table 14. Comparison of brain AChE inhibition at the LOAEL for pups and adults with chlorpyrifos and TCP in blood.

Dosing conditions		Pups		Adults	
		% Inhibition	Chlorpyrifos Blood Level	%Inhibition	Blood Level
Single dose in corn oil	M	51%	(37.5, 234)		--
LOAEL = 5/10*	F	55%	(39.4, 43)	57%	(69.8, 35,3, 15.8, 8.92)
Single dose in milk or	M	42%	(18.2, 25.9)		--
dietary LOAEL = 5/10[&]	F	56%	(40.4, 129)	22%	(0.747, 2.10, 3.24, 4.89)
Repeat dose in corn oil	M	28%	(1.76, 1.82)		--
LOAEL = 1 mg/kg	F	19%	(1.23, 1.95)	9%	(0.237, 0.402, 0.464, 1.07)
3.5 mg/kg	M	68%	(14.4, 7.27)		--
	F	59%	(7.79, 5.86))	69%	(1.65, 2.33, 2.47, 2.37)
			TCP		
Single dose in corn oil	M	51%	(1298, 1807)		--
LOAEL = 5	F	55%	(1973, 2439)	57%	(1824, 2789, 3276, 3325)
Single dose in milk or	M	42%	(1739, 1870)		--
dietary LOAEL = 5	F	56%	(1506, 1791)	22%	(906, 983, 1063, 1796)
Repeat dose in corn oil	M	28%	(589, 827)		--
LOAEL = 1	F	19%	(1191, 1196)	9%	(271, 304, 392, 440)
3.5 mg/kg	M	68%	(827, 589)		--
	F	59%	(1196, 1191)	69%	(1202, 2341, 1739, 1970)

Note : Data for CPY and TCP blood levels are the same as in Table 12 above.
Data are ng/gm for the concentration of chlorpyrifos or TCP
[&] The LOAEL is 5 mg/kg for pups and 10 mg/kg for adults.
-- Not assessed.

Comparison of TCP level in the blood with inhibition of plasma and RBC enzymes following dosing with CPO. Table 15 shows the blood level of TCP and the extent of inhibition at the LOAEL or higher for both plasma and RBC ChE enzymes. Brain AChE was not inhibited by CPO. The association between blood level of TCP and inhibition is clearer for TCP when CPO was dosed than when CPY was dosed. Some implications from Table 15 are as follows:

(Note the symbol ~ indicates that the mean of two values is entered).

-Following an acute dose a correlation may be made for plasma ChE since there was 18% and 21% inhibition associated with ~81 ng/gm for males and ~ 84 ng/gm for females TCP at 0.1 mg/g dose level for males and females. At 0.5 mg/kg there was ~459 ng/gm for males and ~ 317 ng/gm for females associated with 49% for males and 51% for females. This vaguely implies that a lower level may be associated with inhibition in females for plasma ChE. No similar correlation can be made for RBC AChE.

-Comparing the blood level of ~317 ng/gm and 51% inhibition for female pups with the blood level of 725±259 and 56% inhibition for female adults indicates that a lower level of TCP is associated with the same amount of inhibition.
-Following repeat dosing, since no dose effect for inhibition was noted, no correlation between dose level and inhibition can be established.
-The blood level of TCP is similar for both males (~ 153 ng/gm) and female (~136 ng/gm) pups with similar degree of inhibition for plasma (61-62% for both sexes) and RBC AChE (84-86% for both sexes) at 0.5 mg/kg/day dose level.
-For repeat dosing, the blood level of ~136 ng/gm of TCP in female pups was associated with 61% and 86% plasma and RBC enzyme inhibition and a higher level of 300±90 ng/gm was associated with 76% and 87% plasma and RBC enzyme inhibition in adult females at the dose level of 0.5 mg/kg CPO.
-Lower blood levels in female pups following repeat dosing (~136 ng/gm) than following acute dosing (~317 ng/gm) were evident. Also lower blood levels of TCP were evident for repeat dosing of adults (300±90 ng/gm) than for acute dosing (725±259 ng/gm).

Table 15. Correlation between blood level of TCP (ng/gm) and Plasma ChE and RBC AChE inhibition following dosing with CPO.

Dosing	Blood TCP	Plasma ACh Inhibition	RBC AChE inhibition
Single Dose - Pups (124) Males			
0.1 mg/kg	66.4,95.7 (~81)	18%	Ns
0.5 mg/kg	442,475 (~459)	49%	46%
Females 0.1 mg/kg	72,3, 95.9 (~84)	21%	Ns
0.5 mg/kg	340, 293 (~317)	51%	47%
Single Dose – Adult females (135)			
0.5 mg/kg	240, 264,218,198 230±28	56%	36%
1.0 mg/kg	415,655,795,1034 725±259	76%	76%
Repeat Dose – Pups (148) Males			
0.5 mg/kg	133,172 (~153)	62%	84%
Females			
0.5 mg/kg	114,157 (~136)	61%	86%
Repeat Dose – Adult(154)			
0.5 mg/kg	206, 247,351,399 300±90	76%	87%

Data are ng/gm.
CPO analytical data are from Tables 124, 135, 148 and 154 in the study report.

Overall conclusion for the analytical studies.

The above presentation of the analytical data and *attempts* to correlate blood levels of CPY or TCP with inhibition are presented for informational purposes only and no conclusions are being made as to correlate a level of inhibition with a blood level of CPY or TCP. No attempt to correlate these data with a PBPK model for chlorpyrifos is being made at this time.

An important omission for these data are that they do not show if the CPY or TCP blood levels peak before or after the time to peak effect for inhibition. Nor do they show the blood levels when there is noticeable reversal of blood or brain enzyme inhibition.

In addition to the tables with individual analytical data listed above, the report also has several summary tables within the text where comparison of the dose vs. the amount of CPY, CPO or TCP in the blood were made and expressions concerning the increase in blood level for these chemicals relative to the increase in applied dose were made. These discussions are considered by HED to be limited in their value because of the variability in the data.

III. SUMMARY AND CONCLUSIONS

There was good agreement with the assignment of NOAELs and LOAELs between the study author and the HED reviewers. HED reviewers, however, decline from commenting on the extensive application of these data to models as presented in the study report.

Acute exposure. A special *non-guideline* comparative cholinesterase study (2010, MRID No.: 48139301) with CD (Crl:CD(SD) strain rats (8/dose) was conducted to determine the NOAEL and LOAEL for plasma ChE and RBC and brain AChE inhibition following a single oral dose of chlorpyrifos (CPY) and separately for chlorpyrifos oxon (CPO). A *preliminary study* demonstrated the time to peak effect for inhibition of all three enzyme species by CPY in corn oil was 6 hours in pups (at 3 mg/kg) and 8 hours in adults (at 10 mg/kg). Similarly, the times to peak inhibition for CPO in corn oil were determined to be 4 hours for both pups (at 0.5 mg/kg) and adults (at 0.3 mg/kg). The definitive studies consisted of three subparts. In the first subpart, both post natal day (PND) 11 pups and adults rats were dosed by gavage in corn oil. In the second subpart, the PND 11 pups were dosed with CPY in harvested rat milk and the adults were dosed via their diets. In these subparts, CPY doses were 0, 0.05, 0.1, 0.5, 2 or 5 (pups only) or 10 (adults only) mg/kg. In the third subpart, separate groups of PND 11 pups and adults were dosed with 0, 0.005, 0.01, 0.05, 0.1, 0.5 or 1 (adults only) with CPO in corn oil.

The study included assessment of body weight and clinical signs. Differences in these parameters were considered at best threshold and to occur when high levels of AChE inhibition were also evident and to be of lesser significance than the comparison of ChE/AChE inhibition and are not discussed in this Executive Summary.

The following table illustrates the NOAEL and LOAELs derived from the acute dosing aspects of this study. Male pups had the same NOAELs and LOAELs as female pups.

Enzyme Source	Acute NOAEL/LOAEL mg/kg (% Inhibition at LOAEL) Pups (male/female %)	Adults (females only)
Plasma ChE:		
CPY – gavage	0.5/2(51%/47%)	0.5/2(54%)
CPY – milk/diet	0.5/2(39%/44%)	0.5/2(58%)
CPO - gavage	0.05/0.1(18%/21% but 51% at 0.5 mg/kg)	0.1/0.5(56%)
RBC AChE:		
CPY – gavage	0.5/2(35% /31%)	0.5/2(19%)

CPY – milk/diet CPO - gavage	0.5/2(29%/27%)/ 0.1/0.5(46%/47%)	0.5/2(52%) 0.1/0.5(36%)
Brain: CPY – gavage CPY – milk/diet CPO - gavage	 2/5(51%/55%) 2/5(42%/56%) Not inhibited	 2/10(57%) 2/10(22%) Not inhibited

CPY: Comparison of male and female pups. The percent inhibition for male and female pups at the LOAEL was essentially the same for plasma or RBC. When dosed via milk, the male brain was a little less inhibited than females by CPY but the difference (14%) may be assay variation.

CPY: Comparison of pups and adults for inhibition following gavage dosing. The NOAEL for all three enzyme sources for both pups and adults were the same with the brain having a higher NOAEL (2 mg/kg) than for plasma and RBC enzymes (0.5 mg/kg). Quantitative differences at the LOAEL were noted in that the pups (31%) were more inhibited for RBC AChE than adults (19%) at the same dose level. For brain, the pups can be considered more susceptible than adults because although essentially the same degree of inhibition was attained at the LOAEL, the LOAEL is only 5 mg/kg for pups but it is 10 mg/kg for adults.

CPY: Comparison of pups and adults for inhibition following administration via milk to the pups and diet to the adults. Both dosing in milk to pups and via the dietary route to adults resulted in the same NOAEL and LOAELs of 0.5 and 2 mg/kg for plasma and RBC enzymes that was obtained following dosing in corn oil. RBC AChE was more inhibited (52%) in adults than in pups (27%) and the same relationship was apparent for the plasma enzyme. Conversely the brain was more inhibited in female pups (56%) than in adults (only 22%) at the LOAEL even though the LOAELs were 5 mg/kg for pups and 10 mg/kg for adults.

CPO: Comparison of pups and adults for inhibition following gavage dosing. The NOAEL and LOAELs for both plasma and RBC enzymes were established at 0.05 and 0.1 mg/kg. Female plasma ChE demonstrated an apparent lower NOAEL and LOAEL for pups (0.05 mg/kg and 21% inhibition) than for adults (0.1 mg/kg and 56% inhibition) but at the 0.1 mg/kg dose, the pups were only inhibited 51% suggesting that there is no real difference between pups and adults. RBC AChE demonstrated the same LOAEL where the inhibition was comparable (for both pups (47%) and adults (36%). Brain AChE was not inhibited at any dose by CPO.

Repeat Dosing. In the repeat dosing study, PND 11 female pups and adults were dosed with CPY at 0, 0.05, 0.1, 0.5, 1 or 3.5 mg/kg/day for 11 days and plasma ChE and RBC and brain AChE assessments made at 6 hours for pups and 8 hours for adults after the last dose. Similarly, separate groups of PND 11 pups and adults were dosed with 0, 0.01 or 0.5 mg/kg of CPO and assessed 4 fours after the last dose. The study included assessment for clinical sign, body weight and limited FOB and motor activity assessment at the approximate time for peak effect. The following table illustrates the NOAEL and LOAELs derived from the repeat dosing aspects of this study.

Enzyme Source	NOAEL/LOAEL mg/kg (% Inhibition at LOAEL) Pups	Adults
Plasma ChE:		
CPY	0.1/0.5(46%)	0.1/0.5 (46%)
CPO	0.01/0.5 (62%)	0.01/0.5 (76%)
RBC AChE:		
CPY	0.1/0.5 (18%)	0.1/0.5 (20%)
CPO	0.01/0.5 (84%)	0.01/0.5 (87%)
Brain:		
CPY	0.5/1 (19%)	0.5/1 (9%)
CPO	Not inhibited	Not inhibited

CPY: Comparison of pups and adults for inhibition. The same NOAEL and LOAELs resulted for plasma ChE and RBC and brain AChE following repeat dosing. The extent of inhibition at the LOAEL was considered similar at either age.

CPO: Comparison of pups and adults inhibition. Brain enzyme was not inhibited by CPO. The plasma and RBC enzymes demonstrated the same NOAEL and LOAELs of 0.01 and 0.5 mg/kg/day and inhibition at the LOAEL was similar for both enzyme sources.

Analysis of blood for CPY, CPO and TCP. No analytical data for the time to peak effect aspects of this study were presented. The blood samples from the dose response studies were analyzed for CPY, CPO and TCP for only *two* animals of each sex for pups and four adult females. CPO was detected only at higher doses and usually near the level of minimal quantitation. The within group variability of samples often confounded the correlation of blood level of CPY and TCP with inhibition. Therefore, HED reviewers declined from drawing firm conclusions for the blood levels associated with inhibition.

Classification: The classification of this *in vivo* comparative cholinesterase inhibition study is Acceptable/Non-Guideline. The data are considered useful for comparing the pups and adults for *in vivo* inhibition of plasma ChE and RBC and brain AChE. However, the analytical data for CPY and TCP is considered limited in usefulness.

Study deficiencies.

-The study report was very hard to follow with much unnecessary repetition making it hard to mine the document to find critical sought for information.
-The analysis of the milk indicated lower values than their target concentrations and homogeneity was poor in some cases.
-A more serious deficiency is that the analytical data for CPY, CPO and TCP blood levels used only two pups and four adults rendering much variability in the data confounding attempts to correlate blood levels with inhibition or with PBPK models. The analytical data for these chemicals were generated at the time of peak effect only and since neither CPY nor TCP is the active form for inhibition (CPO is), correlation of the blood level with inhibition has only limited value.

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Date: 5/26/11

Date: 5/20/11

DATA EVALUATION RECORD

TXR NO: 0055409

STUDY TYPE: Non-Guideline Acute Inhalation Cholinesterase Study in Rats

DP BARCODE: D380055

P.C.CODE: 059101 (chlorpyrifos)

MRID NO.: 48139303.

TEST MATERIAL (Purity): Chlorpyrifos technical (O,O-Diethyl O-(3,5,6-trichloro-2-pyridinyl)ester phosphorothioic acid, Lot # KC28161419. 99.8%)

SYNONYMS: Chlorpyrifos-ethyl, dursban;

CITATION: J.A. Hotchkiss, S,M. Kreiger, K.A. Brzak and D.L. Dick. Acute inhalation exposure of adult Crl:CD(SD) rats to particulate chlorpyrifos aerosols: Kinetics of concentration dependent cholinesterase (ChE) inhibition in red blood cells, plasma, brain and lung. Toxicology and Environmental Research and Consulting, The DOW Chemical Company, Study No.: 091133, June 29, 2010, MRID No.: 48139303.

SPONSOR: DOW AgroSciences

EXECUTIVE SUMMARY: In this special acute inhalation study (2010, MRID No.: 48139303), groups of 54 adult female rats (Crl:CD(SD)) strain rats were exposed nose only to atmospheric concentrations of 0, 3.7, 12.9, 22.1 or 53.5 mg/m^3 of particulate chlorpyrifos for six hours and allowed an additional 72 hours to recover. The mass median aerodynamic diameter (MMAD)/geometric standard deviations for these exposure levels were determined to be 1.93/1.58, 1.86/1.61, 1.79/1.59 and 1.9/1.51 microns, respectively. Six rats were sampled for plasma, lung, RBC and brain cholinesterase (ChE) at 2, 4 and 6 hours during the exposure (DE) period and at 2, 6, 12, 24, 48 and 72 hours after removal from the exposure chambers (PE) for a total of 9 assessment times. The blood levels of chlorpyrifos, chlorpyrifos oxon and the main metabolite TCP were also analyzed at these times. Urine was collected for analysis of TCP at fixed intervals. The purposes of this study were to generate inhibition NOAELs and LOAELs for each ChE source and to assess for and/or correlate the pharmacokinetic parameters following inhalation exposure to inhibition. A preliminary range finding study described as Phase I assessed chlorpyrifos at 0, 13.3 and 66.7 mg/m^3 and demonstrated that the peak inhibition was at 2 hours for plasma and RBC ChE after removal from the exposure chamber.
ChE data.

Variability of ChE data. There was temporal variability in the control data for plasma, lung and RBC ChE .

Plasma ChE. Dunnet's test demonstrated that the 3.7 mg/m^3 level group is statistically significantly different from the control over time and the response curve shows lower activity over time with indications of reversal toward the end of the study.

Lung ChE. Dunnet's test demonstrated that the 3.7 mg/m^3 exposure group is statistically significantly different from the control over time. From 2 hours during exposure to 12 hours post exposure apparent inhibition ranged from about 20% to about 47% The higher exposures indicated a dose response with maximum inhibition at the expected times.

RBC ChE. Dunnett's test demonstrated statistical significance at the 12.9 mg/m^3 ranging from about 30% to about 80% inhibition. The top three exposures demonstrated a reasonably clear dose response.

Brain ChE. Dunnett's for the group over all time points was determined to be statistically significant for the highest exposure level only. Pair wise comparisons of brain ChE assessments at the nine assessment times did not reach statistical significance although decreases in activity were as much as about 24%.

Conclusion for inhibition of ChE. **The LOAEL for ChE inhibition is 3.7 mg/m^3 based on lung and plasma ChE. A NOAEL was not established.**

Pharmacokinetic data.

Achieved dosage: Based on urinary excretion of TCP, the principal metabolite of chlorpyrifos, the absorbed doses of chlorpyrifos were 0.52, 2.86, 2.21 and 5.7 mg/kg for the 3.7, 12.9, 22.1 and 53.5 mg/m^3 atmospheric exposure levels, respectively. It is noteworthy that the absorbed dose for the 12.9 mg/m^3 exposure is higher than for the 22.1 mg/m^3.

Chlorpyrifos. The blood level of chlorpyrifos peaked at 4 or 6 hours DE and rapidly declined PE. At the end of the six hour exposure time, the blood level of chlorpyrifos was 5.7±2.4, 34.7±5, 28.7±7.5 and 59.1±22.5 ng/gm for the 3.7, 12.9, 22.1 and 53.5 mg/m^3 exposure groups, respectively.

Blood TCP. The blood level of TCP peaked at 12 (for the high exposure group) and at 24 hours post exposure for the other exposure groups. At their peaks, the blood levels of TCP were 301.3±110.6, 1110.1±103.2, 1166.4±585.3 and 2439.4±598.6 ng/gm for the control to high exposure groups, respectively.

Urinary TCP. Assessment of urinary TCP levels demonstrated a half-life for TCP of approximately 11 hours for all four exposure levels. Peak urinary TCP levels were at the 0-12 hour collection PE collection period. Total TCP as chlorpyrifos equivalents were 123.09±17.34, 669.25±65.9, 503.68±99.66 and 1354.32±356.61 μg equivalents of chlorpyrifos. Consistent with

the blood levels, the 22.1 mg/m^3 exposure level did not demonstrate an intake consistent with the progression of exposure concentrations.

Chlorpyrifos oxon. The blood level of chlorpyrifos oxon was transient and usually below the level of quantitation (0.1 ng/gm). It was present at the highest exposure level (and occasionally at lower levels) at up to 0.22 ng/gm during the exposure period or at 2 hours after exposure (0.12 ng/gm).

Conclusions for pharmacokinetic data. There is poor correlation between the 12.9 and 22.1 mg/m^3 exposure groups for all pharmacokinetic parameters with the lower nominal level of 12.9 mg/m^3 consistently showing more internal exposure than the 22.1 mg/m^3 level. No other conclusions for the pharmacokinetic data will be made at this time. This DER presents only an indication as to what pharmacokinetic data are available in this study.

CLASSIFICATION: The classification of this non-guideline special inhibition study is Acceptable/Non-Guideline. The study is considered to have useful data. The study contains pharmacokinetic data to help further characterize the uptake and elimination of chlorpyrifos and its principal metabolite TCP.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. There was no Flagging page provided.

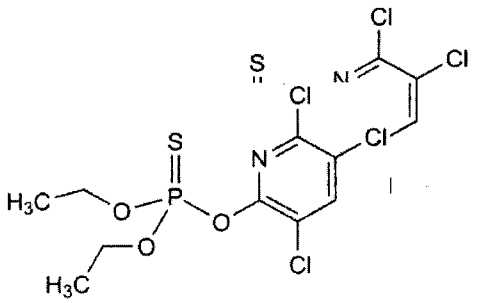
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

Description: Chlorpyrifos technical (no physical description provided)
Lot/batch #: (lot# KC28161419, TSN101285).
Purity: 99.8% a.i.
CAS # of TGAI: 2921-88-2

Structure:



O,O-Diethyl O-(3,5,6-trichloro-2-pyridinyl)ester phosphororthioic acid.

2. Vehicle and/or positive control: No vehicle or positive control in this study.

3. Test animals: (page 26)

Species: Rat (females only).
Strain: CD rats (Crl:CD(SD))
Age/weight at dosing: 12 weeks/ Weight not provided and no body weight tables provided.
Source: Non-cannulated rats: Charles River Laboratories Inc. Raleigh, NC
Cannulated rats (indwelling jugular catheters): Taconic Laboratories, Germantown, New York
Housing: One per cage. Also special conditions for nose only exposure.
Diet: LabDiet Certified Rodent Chow #5002 (PMI Nutrition). *(ad libitum)*
Water: Municipal *(ad libitum)*
Environmental conditions:
Temperature: 22° ± 1° (maximum ± 3° C)
Humidity: 40-70%
Air changes: 12-15 times/hour (while not in inhalation chamber)
Photo period: 12 hr light/12 hr dark (on 6 am, off 6 pm)

B. STUDY DESIGN:

1. In life dates: Start: January 8, 2010; End: cannot find the exact date but final report is dated June 29, 2010.

2. Animal assignment and treatment and achieved dose: Animals were assigned to the test groups noted in DER Table 1.

DER Table 1. Study design[1], chamber concentration, achieved chlorpyrifos dosage, MMAD and GSD.

Group	Animals	Chamber mg/m^{3}[a]	MMAD/GSD (microns)
Pilot Study – Phase 1 (with cannulated rats)			
Control	6♂ /6♀	--	--/--
Low	"	13.3 (7.4-19)[b]	1.40/2.12[c]
High	"	66.7 (42-100)	1.44/1.39
Definitive Study – Phase 2			
Control	6 ♀/assay time	--	--
Low	"	3.7 (3.2-4.4)[d]	1.93/1.58[e]
Mid -1	"	12.9 (9.4-15)	1.86/1.61
Mid - 2	"	22.1 (21-23)	1.79/1.59
High	"	53.5 (37-63)	1.91/1.51

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[1]For the definitive study, assessments for plasma, lung, RBC and brain ChE were made at 2, 4 and 6 hours (hr) during exposure (DE) in the chambers and 2, 6, 12, 24, 48 and 72 hr after removal from the chamber (PE). Blood samples were also taken for chlorpyrifos, chlorpyrifos oxon and TCP analysis at each time point. Cumulative urine samples were also taken (6 rats) for six hours during exposure and hours 0-12, 12-24, 24-48 and 48-72. For the Pilot Study, samples were taken at 2 and 4 hours during exposure and 0.5, 1, 2, 4, 6, 12 and 24 hr PE. The blood serum (150 µl) was pooled from the same two rats for each assessment time.
[a] Time Weighted Average (TWA), the range of values is presented in the (). Appendix 12, page 98 for the definitive study. [b] Appendix 1, page 79. [c] Appendix 2, page 80.
[d] The number on the first line is the theoretical dose in mg/kg obtained calculated by assuming a relative minute volume of 0.78 L/min-kg body weight. The number in the second line is the achieved dose based on the analytical data for TCP (Study report Table 3, page 40 and of this DER for discussion).
[e] Appendix 13, page 99.

3. **Generation of the test atmosphere and chamber conditions.**

For the first phase of the study, the test material was first ground using a "blade type coffee mill" and the resulting grind was subjected to size selection by means of stainless steel sieves. Particles of less than 125 microns in diameter were fed to a Jet Mill (Model 00 Jet-O-Mizer, Fluid Energy Aljet, Plumsteadville, Pennsylvannia) for final size reduction and then delivered to the exposure chamber. For the second or definitive phase of the study, the stock test material was described as being cyromilled at Howard Industries (Columbus, Ohio). It was reported that the milled test material tended to clump during storage, and therefore it was dispersed by passage through stainless steel sieves. The sieved material with a geometric diameter of less than 75 microns was then delivered to the exposure chamber by means of a Wright Dust generator (BGI Inc. Waltham, MA). The report states that "various glassware and cyclones were placed between the dust generators and the chamber to achieve a particle size of less than 2 microns. The Mass Median Aeroydynamic Diameter (MMAD) and Geometric Standard Deviation data as reported in the study report are presented in Table 1 above.

There was limited description of the exposure chambers (p. 19) that were described as being of 42 and 53 liters volume for the phase I and II studies, respectively. There was no description of the means to attain nose-only exposure or how the chambers and tubes were modified to collect urine during the exposure period in the original study report. Subsequently additional information on the chambers was submitted via e-mail (K. Racke to Y. Donovan, January 16, 2011).

4. In-Life Assessments.

Daily cage side examinations were made once daily. No data on clinical signs or body weight were presented. It is assumed that no marked effects of exposure resulted or at least the study does not present an assessment of reactions to treatment following inhalation exposure.

5. Post Mortem Assessments.

Aside from sampling the lung and brain, no descriptions of post mortem assessments were provided.

6. Plasma ChE and lung, RBC and brain ChE. Phase 1 (cannulated rats): Approximately 150 µl/rat/sample of blood was taken from the cannula at the prescribed times. The plasma and RBCs were separated and frozen and shipped to the WIL Laboratory for analysis. It was necessary to combine the blood sample from two rats for plasma in order to have enough for the plasma ChE assay. No lung or brain samples from Phase 1 were analyzed.

Phase 2. The rats were sacrificed at predetermined time to peak effect following deep anesthetization by CO_2/O_2 inhalation. Blood was collected from the heart by cardiac puncture. For Phase 2 of the study, the lungs (including the left and right lobes and extrapulmonary bronchi) and brain were removed and snap frozen in liquid nitrogen. For the analysis of enzymes, the blood sample was reported to be kept on ice, centrifuged to separate the plasma from the RBCs. The RBCs were diluted 1:20 with 1% Triton X-100. Both plasma and lysed RBCs were frozen at -80°C and the lung and brain samples were shipped to the WIL Laboratory for analysis of ChE and ChE. The protocol or SOP (effective date September 12, 2006) that the WIL Laboratories used for assessment of ChE and ChE was provided to HED upon request from the reviewer.

7. Samples for analysis of chlorpyrifos, chlorpyrifos oxon and TCP. Blood samples from Phase 2 were taken from *four* animals at each time point. Cumulative urine samples were taken periodically (see Table 1).

8. Statistics (from page 29). Statistical analysis was conducted by a two-way analysis of variance with exposure level (dose), time and the interaction between exposure level and time as the model. If the dose effect was significant, least square means were calculated. The control mean was compared to each exposure concentration group mean and a Dunnett's test correction applied. If the dose by time effect was significant, all pair wise comparisons of the least square means were conducted and the Tukey's correction applied. The comparisons reported for the interaction were the control mean to the individual exposure concentration group means at each time point. Significance level was set at "alpha=0.05.

Additional statistical analysis of the ChE/ChE data using a one-way analysis of variance to determine intergroup differences was conducted at the WIL Laboratory where the data were generated (presented in Appendix A of the report). The study report, however, relied on the two-way analysis of variance as described above.

III. Results

A. Clinical signs and body weight.

Although the methods section indicated that cage side assessment for clinical signs were made, no comments or data were presented in the results section. No information on body weight was presented. ***Reviewer's note:*** Since this is an acute study, and the emphasis on assessment of ChE and ChE inhibition and the pharmacokinetics of chlorpyrifos following inhalation exposure, the assessment of clinical signs would be be helpful to interpretation of the study. It is assumed that there were no consistent effects on clinical signs or bodyweight.

B. Plasma, Lung, RBC and Brain ChE.

1. ChE dose-response data.

Plasma, RBC and brain ChE. "Table 5" (two pages, transferred from pages 60 and 61of the final study report) illustrates the mean values for the plasma, lung, RBC and brain ChE data sets for the dose response part of the study. The relative relationship of plasma ChE (2137 ±814.5 (38%)) < RBC ChE (4942.3±482 .8 (9.8)) < brain ChE (51934±1801.2 (3.5%)) is typical for chlorpyrifos (units are U/L mean and standard deviation for the control group at 2 hours during dosing). Lung ChE (3361.5±556.7 (16.6%) U/L) is not usually assessed for comparison in this sequence.

Variance. Examples of the variance (standard deviation as % of the mean, also show in the above section) for plasma, lung, RBC and brain ChE control groups over time for the 9 time samplings* in the definitive study are as follows:

Plasma ChE: 20% (6 hr DE) to 56% (4 hrs DE)

Lung ChE: 8% (4 hr DE) to 24% (2 hr PE)

RBC ChE: 8.5% (24 hr PE) to 19.8% (72 hr PE)

Brain ChE: 3.1% (48 hr PE) to 19.1% (24 hr PE)

Consistency over the time course of the study. Across the 78 hour time course in the definitive study each enzyme source for the untreated control group varied from a low to a high value as follows (data in U/L):

Plasma ChE: 1905.2±449.7 at 2 hr (PE) to 2904.8±703.5 at 48 hr PE. (1.52 fold)
[1][Phase 1 (females): 2801.7±514.6 at 24 hr PE to 3791±782.2 at 6 he DE. (1.35 fold)]
[Phase 1 (males): 938.3±183.7 at 12 PE to 1106.7±186.1 at 4 hr DE. (1.18 fold)]

Lung ChE: 2985.8±662.6 at 12 PE to 4453.2±1013.6 at 6 hrs DE (1.49 fold).

RBC ChE: 2346±388.8 at 6 hr PE to 4942.3±482.8 at 2 DE (2.11 fold)
[1][Phase 1 (females): 4955.2±351.4 at 24 hr PE to 7577.2±4604.6 at 12 hr PE. (1.53)]
[Phase 1 (males): 4380±609.9 at 6 hr PE to 6411.3±1891.1 at 2 hr DE (1.46 fold)]

Brain ChE: 46603.5±7672.8 at 6 hr DE to 52866.7±4121 at 6 hr PE (1.13 fold)

[1] Data from the phase I study for both sexes for both plasma and RBC enzymes where the PE times was up to 24 hours (0.5, 1,2,4,6, 12 and 24 hours) are also included for comparison. Lung and brain ChE were not assessed in phase I.

2. Phase I (Pilot study) –

The results of the pilot study are presented in Tables 2 and 3 of the study report and attached. Maximum inhibition occurs at 2 hours following removal from the exposure chamber. Evidence for substantial inhibition was attained at the exposure level of 13.3 mg/m^3 starting during exposure (i.e.52% apparent inhibition at 6 hr DE) for RBC ChE and up to 85% apparent inhibition for plasma ChE at 6 hr DE). At 2 hours PE, RBC ChE was inhibited to 80.2% and plasma ChE was inhibited to 84%.

The study author maintained that for both exposure levels the females were "generally inhibited to an equal or greater degree compared to male rats. This would justify the selection of females only for the definitive study.

3. Phase 2 - Definitive study.

Note: For Sections 4, 5, 6 and 7 below, please refer to Table 5 (mean and standard deviation in actual data in U/L- 2 pages) and 6 (normalized activity as percent of control- 2 pages) for a total of four pages that were reproduced from the final study report.

4. *Plasma ChE inhibition.*

Figure 4 (appended from study report) depicts plasma ChE vs time. Overall by the using the Dunnet's test, the 3.7 mg/m^3 exposure group is statistically significantly different from the control supporting inhibition. A statistically significant lower value (48%, $p < 0.05$) was noted in the low (3.7 mg/m^3) exposure group at 6 hr DE.

The study report (see page 36) indicates that there is inhibition at 3.7 mg/m^3 and that there is increased inhibition at the higher exposures to demonstrate a dose response. Support for a conclusion that there is inhibition at 3.7 mg/m^3 is evident by the pattern of activity across time since the 2 hr DE assessment is higher than subsequent times and is lowest at the expected time of peak inhibition (2 hr PE) and later begins to return to higher levels following a pattern considered consistent with inhibition at higher levels.

The LOAEL for plasma ChE inhibition in adult female rats as 3.7 mg/m^3 and the NOAEL is not established.

5. ***Lung ChE inhibition.***

Similar to the plasma ChE data, the 3.7 mg/m^3 exposure group is reported as being statistically significantly lower according to Dunett's test.

DER Table 2. Comparison of lung ChE inhibition data at 3.7, 12.9 and 22.1 mg/m^3 and blood level of chlorpyrifos.

Interval	Control	3.7 mg/m^3[a]	12.9 mg/m^3	22.1 mg/m^3
2 DE	3361.5±556.7	2489.3±280.6 26% -- 2.6±0.1	2279.5±292.9 32% -- 8.3±3.1	2718.8±1048.4 19% -- 16.3±4.2
4 "	3107.2±258.8	2431±528 22% ~0% 4.3±0.5	2258.2±971.5 27% ~0% 31.9±9.6	1470.2±366.4 53% 46% 33.9±10
6 "	4453.2±1016.6	2347.3±377.1 47%* ~0% 5.7±2.4	1263.8±213.1 72% 45% 34.7±5	1701.2±1258.9 62% 37% 28.7±7.5
2 PE	3942.2±956.4	2391.2±339.5 39%* 4%	1062.7±185.2 73% 53%	1688.3±848.2 57% 53%
6 "	3070.7±310	2337.3±469.7 24% ~4-5%	1440.8±271.9 53% 37%	1048.8±384.3 66% 61%
12 "	2985.8±662.6	2399.7±448.3 19.6% ~4%	1330.2±361.3 55% 42%	2235.7±1672.9 25% 18%
24 "	3002.2±452.5	3818.8±1638 +27% +45%	2001.5±833.5 33% 12%	1684.8±229.3 44% 38%
48 "	3132±426	2920±107.6 7% +17%	2266.5±258.5 28% ~0%	2361.8±336.8 25% %
72 "	3087±308.7	2838.2±253.8 8% +14%	2762.5±543.4 11% +21%	2462.7±356.3 20% 9%

[a] The entries are: top line - mean and standard deviation for the lung ChE; next line – percent lower than the control group and percent different from 2 hour reading; third line the blood level of chlorpyrifos at the assessment time (blood levels were minimal after exposure stopped and not included.) The high exposure group where there is substantial inhibition are not included here because this table is meant distinguish differences (i.e. inhibition) between the low exposure group and from the control and compare with the next two higher doses.

Figure 1 displays the lung data for the control, 3.7 and 12.9 mg/m^3 exposure groups for up to 24 hours PE. The higher doses and later times were not included to reduce the clutter in the plot so that the differences between the control and the two exposure groups can be better seen.

DER Table 2 and Figure 1 (appended) indicate that the pattern of activity for lung ChE across time at 3.7 mg/m^3 is very constant.

The LOAEL for lung ChE inhibition in adult female rats is 3.7 mg/m^3 and the NOAEL is not established.

6. ***RBC ChE inhibition***

Unlike the plasma and lung data, the 3.7 mg/m^3 group did not show statistically significance difference by Dunnett's test. Dunnett's test was significant at 12.9 mg/m^3 and above.

At 12.9 mg/m^3 there is t evidence of inhibition.

The NOAEL for RBC ChE inhibition in adult female rats as 3.7 mg/m$^{3.}$ The LOAEL is 12.9 mg/m^3.

It is noted that the pilot study demonstrated that at the slightly higher level of 13.3 mg/m^3 with apparent RBC ChE inhibition ranging to ~80% (at 2 hr PE) confirming the inhibition at 12.9 mg/m.

7. ***Brain ChE inhibition***

Dunnett's test indicated statistical differences at the high exposure only. However, none of the pair wise comparisons were shown to be statistically significant even at the highest exposure of 53.5 mg/m^3. It is noted that the standard deviations for many of the means were high such as at 6 hr DE for the 12.9 mg/m^3 when it was 33% and at 24 hr PE for the 22.1 mg/m^3 group when it was 46%.

At 53.5 mg/m^3 the decreases in brain ChE activity, although not showing statistical differences ranged from "25 to 24% reduction in brain ChE activity, compared to the 0 mg/m^3-exposed control rats" (p. 37). HED reviewers note that one of the greatest decrease ~24% was at 72 hr PE and this time is well past the expected time for peak inhibition.

The NOAEL for brain ChE is 22.1 mg/m^3 and the LOAEL is 53.5 mg/m^3.

8. ***Summary of cholinesterase inhibition.***

The LOAEL is 3.7 mg/m^3 based on plasma and lung ChE inhibition. A NOAEL was not established.

C. Analysis of Blood for Chlorpyrifos, chlorpyrifos oxon and TCP

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Note-This section is being prepared to indicate the types of analytical data available in the study report with comments on their variability and no attempts to interpret these data in pharmacological models are being made at this time.

1. ***Reliability of the analytical data. Table 7 from the study report conveys the analytical data that will be discussed below.***

Chlorpyrifos oxon. The limit of detection for chlorpyrifos oxon was 0.1 ng/gm. Chlorpyrifos oxon was detected only in a few samples and the highest level recorded was 0.22 ±0.10 ng/gm at 2 hr DE for the 53.5 mg/m^3 exposure group. The high exposure group had detectable levels at 2, 4 and 6 hr DE and 2 hr PE (0.12 for just one sample). The 12.9 and 22.1 mg/m^3 exposure groups also had detectable oxon but only during the exposure period. RAB V considers that the chlorpyrifos oxon analysis only confirms that its presence in blood is transitory and detectable at higher exposures. Thus, blood levels of chlorpyrifos oxon are of lesser value than parent chlorpyrifos and the principal metabolite TCP.

Chlorpyrifos. The lower limit of detection for chlorpyrifos was 0.1 ng/gm. The highest levels of chlorpyrifos were found during the exposure period. The mean values and variance for each exposure group at 4 or 6 hr DE a time when the highest concentration was attained are as follows shown in DER Table 3. As indicated, the variability is high at all exposure levels based on the high variance (29 to 45%).

TCP. The lower limit of detection for TCP was 10.2 ng/gm. TCP levels peaked at 24 hours for the three lower exposures but at 12 hours for the highest exposure group. Mean and variance values for each exposure group are shown in DER Table 3. The variability ranged from 9.3% to 50.2%.

DER Table 3. Variability of the chlorpyrifos and TCP data in blood at peak levels.

Exposure mg/m^3	Chlorpyrifos		TCP (ng/gm)	
	ng/gm±SD	Variance at time	ng/gm±SD	Variance at time
3.7	5.7±2.4	42% - 6 hr DE	301.3±110.6	37% - 24 hr PE
12.9 (3.5)[a]	31.9±9.6 (6.1)	30% - 4 hr DE	1101.1±103.2 (3.7)	9.3% - 24 hr PE
22.1 (6)	33.9±10 (5.9)	29% - 4 hr DE	1166.4±585.3 (3.9)	50.2% - 24 hr PE
53.5 (14.5)	64.8±29.2 (11.4)	45% - 4 hr DE	2439.4±589.6 (8.1)	26.2% - 12 hr PE

(The examples in this table are at the peak blood level and variance may be higher at other time points.)
[a] The number in () is the exposure multiple relative to the low dose.

Overall comments on the variance associated with the analysis of chlorpyrifos and TCP in blood. The analytical results tend to show large standard deviations and associated variance and this variability needs to be taken into consideration before conclusions on the significance of levels of chlorpyrifos and/or TCP are correlated with inhibition or with PBPK models.

The blood levels of both chlorpyrifos and TCP for the 12.9 and 22.1 mg/m^3 exposure groups are very similar although there is nearly a twofold difference in exposure. For example, there is a progression of 3.5, 6, and 14.5 multiples (column 1) between the exposure levels of 3.7, 12.9, 22.1 and 53.5 mg/m^3 levels. These multiples are 6.1, 5 and 11.4 for the blood levels of chlorpyrifos (column 2). The 12.9 mg/m^3 group seems too high (compare the multiples 3.5 for exposure level and 6.1 for analytical level for chlorpyrifos). The 22.1 mg/m^3 exposure group seems more consistent with the expected multiple, compare 6 and 5). Based on TCP levels, the 12.9 mg/m^3 exposure group (compare multiples of 3.5 and 3.7) is more consistent with the expected value than for the 22.1 mg/m^3 exposure group (contrast multiple of 6 and only 3.9).

2. Correlation of exposure, inhibition and chlorpyrifos at the end of the exposure time (6 hr DE).

As an exercise in attempting to correlate the blood level of chlorpyrifos with inhibition the blood level of chlorpyrifos at 6 hr DE is compared with the apparent inhibition as follows for plasma, lung and RBC enzymes as shown in DER Table 4.

DER Table 4. Correlation of blood level of chlorpyrifos with inhibition of plasma ChE, lung or RBC ChE at 6 hr DE.

Exposure (mg/m^3)	Chlorpyrifos (ng/g)[a]	Plasma ChE Activity[1] (~inhibition)	Lung ChE Activity[1] (~inhibition)	RBC ChE Activity[1] (~inhibition)
3.7	5.7±2.4	52.1±20.6	52.7±8.5	137.3±29.4
12.9	34.7±5	(~48%)	(~47%)	(+~37%)
22.1	28.7±7.5	23.1±4.9	28.4±4.8	59.8±14.8
53.5	59.1±22.5	(~77%)	(~72%)	(~40%)
		25.6±5.5	38.2±28.3	31.3±25.1
		(~74%)	(~62%)	(~69%)
		30.8±11.0	25.2±10.2	25.2±18.3
		(~79%)	(~75%)	(~75%)

[1] Normalized activity expressed as % relative to the control ± standard deviation (estimated percent inhibition calculated as 100 - activity).
+ indicates an increase in activity rather than inhibition.

The inhibition of RBC enzyme at higher doses shows at least some dose response with exposure level. However, since the blood levels of chlorpyrifos are similar for both the 12.9 and 22.1 mg/m^3 exposure groups, these blood levels would predict a similar level of inhibition and not a higher level for the 22.1 (69%) than for the 12.9 (40%) mg/m^3 exposure groups.

Blood levels of TCP. Study report Table 7 and DER Table 3 indicate that the TCP peaks in the blood at 24 hours or 12 hours PE. The study report (p. 38) states "The substantial increase

in blood TCP levels post exposure, compared to the rapid decline in blood CPF (chlorpyrifos) levels, indicates that substantial pre-systemic metabolism of CPF to TCP occurs in the upper and lower respiratory tract." RABV reviewers do not concur with this explanation on the basis of available information. Additional verification is needed.

D. Analysis of Urine for TCP.

Table 11 (pages 71-74) of the study report presents individual and mean data for collection times and intervals (0-12, 12-24, 24-48 and 48-72 hour) for each exposure level (one page per exposure level) for:

-amount of TCP per interval (µg)
-cumulative TCP (µg)
-cumulative TCP as CPF equivalents (µg)
-urinary elimination rates (µg TCP/hr) and half life.

The results are summarized in DER Table 5.

DER Table 5. Urinary TCP data. (Pages pages 71-740).

Exposure mg/m^3	Half life hr	Peak Urinary 0-12 hr, µg	Cumulative 72 hr, µg	TCP as Chlorpyrifos	Comparison Pred/Obt[b]
3.7	11.5±1.41	31.08±8.91	52.08±7.34	123.09±17.34 (0.52±0.07[a])	--/--
12.9	11.57±1.67	141.11±16.11	283.14±27.88	669.25±65.9 (2.86±0.25)	3.5/5.43 (5.5)
22.1	10.58±1.75	127.63±24.93	213.09±42.17	503.68±99.66 (2.21±0.41)	6/4.09 (4.25)
53.5	10.71±1.62	336.9±101.65	572.98±150.87	1354.32±356.61 (5.70±1.48)	14.5/11 (11)

[a] The absorbed dose as calculated by the study author (page 40) using the formula "TCP(mg/kg/body wt.) x (198 g/mol TCP/351 g/mol CPY g/mol)/0.75 in mg/kg".
Reviewer's note: This formula as expressed on page 40 and discussed on page 39 in the study report renders a value of about 1.81 mg/kg for the high dose (TCP mg/kg = 2.41) does not result in the value for absorbed dose of CPY reported. Inverting the molecular weights for TCP and CPY renders the values listed in the author's table. Inverting makes more sense because TCP is a part of the CPY molecule and there would be a higher number for the entire molecule. E-mail correspondence with the study author (3/23/11) confirmed that the correct form of the equation should have the MW of chlorpyrifos as the numerator and the MW of TCP as the denominator.
[b] The "Pred" (predicted) progression of the dose is compared based on the nominal exposure levels (first column) for relative to the lowest exposure to highest exposure. The "Obt" progression compares the lowest dose with the higher doses for TCP as chlorpyrifos and for absorbed dose in ().

Calculating the cumulative TCP and CPY equivalents relative to the value for the 3.7 mg/m^3 exposure (as unity) results in a progression of 4.95, 4.08 and 11. The progressions for the 12.9 and 22.1 mg/m^3 did not maintain an internal dose consistent with the progression.

IV. Discussion/Conclusions.

ChE data. The stated purpose (i.e. p. 40) of the study was "The study was designed to provide essential data on the absorption and metabolism of inhaled particulate chlorpyrifos (CPF) aerosols and the kinetics of ChE inhibition resulting from inhalation exposure to CPF." The study report asserts (p. 41) that the "rank order of inhibition between tissues (relative to controls) of RBC $\geq$ Plasma $\approx$ Lung $\gg$ Brain" (p. 41). The report also states that "inhibition of plasma and lung ChE was statistically identified in all exposure levels, Dose dependent RBC ChE activity was statistically identified in only the three highest exposure groups." The report also concluded that the "NOEC" for brain ChE inhibition is 22.1 mg/m^3 and that there is inhibition at 53.5 mg/m^3.

HED concludes that the LOAEL for this study is 3.7 mg/m^3 based on inhibition of plasma and lung ChE. No NOAEL was established. RBC ChE was inhibited at 12.9 mg/m^3 and above. Brain ChE was inhibited at 53.5 mg/m^3, the highest dose tested.

Pharmacokinetic data. The study report discusses the analysis of chlorpyrifos and its oxon in blood and asserts that the peak level of chlorpyrifos occurs during the exposure and declines rapidly afterwards. TCP peaks during the post exposure period at 12 or 24 hours. The half-life of chlorpyrifos based on urinary content of TCP was determined to be about 11 hours for all four exposure levels.

V. Study Deficiencies.

There was an inconsistency between the internal exposures for the 12.9 and 22.1 mg/m^3 exposure groups because the internal dose for the 12.9 mg/m^3 exposure group was slightly higher than the 22.1 mg/m^3 exposure group. The source of this difference is not known. The study author's suggestion of differences in the minute inhalation volume is considered speculative.

ACUTE INHALATION EXPOSURE OF ADULT CRL:CD(SD) RATS TO PARTICULATE CHLORPYRIFOS AEROSOLS: KINETICS OF CONCENTRATION-DEPENDENT CHOLINESTERASE (ChE) INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG

TABLE 2. Red Blood Cell (RBC) and Plasma Cholinesterase (ChE) Activity Summary Table – Phase 1

Sex	Exposure		Cholinesterase Activity (U/L) During Exposure 2	4	6	After Exposure 0.5	1	2	4	6	12	24
RBC												
Male	0 mg CPF/m³ (Control)	Mean	6411.3	5627.3	5632.0	5206.8	5368.0	5256.4	5391.6	4380.0	5344.0	4846.4
		S.D.	1891.1	1083.7	809.9	823.3	1555.4	1157.8	884.9	609.9	628.7	529.6
	13.3 mg CPF/m³ (Low) *	Mean	4788.7	4562.0	2706.0	2310.3	2430.3	1859.0	2229.0	2601.0	3316.7	3171.3
		S.D.	1563.8	1292.8	923.8	790.0	895.2	527.5	380.2	961.2	1467.0	1289.4
	66.7 mg CPF/m³ (High) *	Mean	2545.7	887.7	270.3	181.0	164.3	19.0	156.0	210.3	673. 7	947.0
		S.D.	615.5	388.1	143.5	105.6	168.7	22.0	133.9	307.6	336.4	302.6
Female	0 mg CPF/m³ (Control)	Mean	6517.0	6760.0	6505.3	5950.0	6068.8	5917.6	7277.2	5254.0	7577.2	4965.2
		S.D.	943.0	1947.5	1617.5	1677.8	1118.5	1049.6	3215.2	579.1	4604.6	351.4
	13.3 mg CPF/m³ (Low) *	Mean	4793.3	**3344.3 $**	**1672.0 $**	**1608.4 $**	**1403.3 $**	**1173.3 $**	**1555.0 $**	**1467.7 $**	**2440.7 $**	3684.3
		S.D.	1415.6	**1508.4**	**713.2**	**734.5**	**387.4**	**380.0**	**1151.3**	**579.3**	**649.9**	1219.6
	66.7 mg CPF/m³ (High) *	Mean	**1893.3 $**	**510.7 $**	**204.3 $**	**287.0 $**	**24.8 $**	**15.2 $**	**76.8 $**	**70.8 $**	**363.2 $**	**592.4 $**
		S.D.	**720.9**	**327.0**	**114.2**	**122.4**	**20.9**	**11.6**	**128.2**	**83.8**	**241.3**	**496.9**
Plasma												
Male	0 mg CPF/m³ (Control)	Mean	1086.3	1106.7	1103.0	1093.3	1084.3	1062.7	995.0	1035.0	938.3	1045.0
		S.D.	177.2	186.1	218.7	152.5	160.5	155.0	148.5	148.3	183.7	129.5
	13.3 mg CPF/m³ (Low) *	Mean	931.3	**568.0 $**	**405.3 $**	**372.0 $**	**383.0 $**	**398.7 $**	**482.3 $**	**527.0 $**	644.0	780.0
		S.D.	109.6	**43.6**	**51.2**	**66.6**	**67.0**	**70.3**	**67.0**	**52.7**	33.2	71.8
	66.7 mg CPF/m³ (High) *	Mean	**410.3 $**	**184.0 $**	**125.3 $**	**126.7 $**	**122.0 $**	**125.0 $**	**165.7 $**	**200.3 $**	**341.7 $**	**560.3 $**
		S.D.	**42.4**	**25.5**	**5.5**	**5.9**	**13.5**	**11.3**	**25.7**	**37.6**	**39.5**	**39.3**
Female	0 mg CPF/m³ (Control)	Mean	3699.7	3622.3	3791.0	3606.3	3579.0	3448.3	3428.0	3273.0	2973.3	2801.7
		S.D.	661.1	991.9	782.2	685.6	623.6	618.9	759.4	605.2	494.5	514.6
	13.3 mg CPF/m³ (Low) *	Mean	1206.7	682.3	550.0	467.7	512.0	540.3	642.0	734.3	972.0	1235.7
		S.D.	226.7	55.5	65.6	109.6	97.8	98.2	149.3	210.2	310.1	271.6
	66.7 mg CPF/m³ (High) *	Mean	700.0	366.0	278.0	268.7	265.7	233.3	290.3	280.0	301.7	283.7
		S.D.	127.3	59.0	14.7	51.1	23.5	7.2	47.7	76.4	46.1	26.6

* DOSE LEAST SQUARE MEAN STATISTICALLY DIFFERENT FROM CONTROL BY DUNNETT'S TEST, ALPHA=0.05.
BOLD = $ DOSE*TIME LEAST SQUARE MEAN STATISTICALLY DIFFERENT FROM CONTROL DOSE*TIME BY TUKEY'S TEST, ALPHA=0.05

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ACUTE INHALATION EXPOSURE OF ADULT CRL:CD(SD) RATS TO PARTICULATE CHLORPYRIFOS AEROSOLS: KINETICS OF CONCENTRATION-DEPENDENT CHOLINESTERASE (ChE) INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG

TABLE 3. Normalized Red Blood Cell (RBC) and Plasma Cholinesterase (ChE) Activity (% of Control) Summary Table – Phase I

Sex	Exposure		Normalized Cholinesterase Activity (% of Control)									
			During Exposure			After Exposure						
RBC			**2**	**4**	**6**	**0.5**	**1**	**2**	**4**	**6**	**12**	**24**
Male	0 mg CPF/m³ (Control)	Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		S.D.	29.5	19.3	14.4	15.8	29.0	22.0	16.4	13.9	11.8	10.9
	13.3 mg CPF/m³ (Low) *	Mean	74.7	81.1	48.0	44.4	45.3	35.4	41.3	59.4	62.1	65.4
		S.D.	24.4	23.0	16.4	15.2	16.7	10.0	7.1	21.9	27.5	26.6
	66.7 mg CPF/m³ (High) *	Mean	39.7	15.8	4.8	3.5	3.1	0.4	2.9	4.8	12.6	19.5
		S.D.	9.6	6.9	2.5	2.0	3.1	0.4	2.5	7.0	6.3	6.2
Female	0 mg CPF/m³ (Control)	Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		S.D.	14.5	28.8	24.9	28.2	18.4	17.7	44.2	11.0	60.8	7.1
	13.3 mg CPF/m³ (Low) *	Mean	73.6	**49.5**	**25.7**	**27.0**	**23.1**	**19.8**	**21.4**	**27.9**	**32.2**	74.2
		S.D.	21.7	**22.3**	**11.0**	**12.3**	**6.4**	**6.4**	**15.8**	**11.0**	**8.6**	24.6
	66.7 mg CPF/m³ (High) *	Mean	**29.1**	**7.6**	**3.1**	**4.8**	**0.4**	**0.3**	**1.1**	**1.3**	**4.8**	**11.9**
		S.D.	**11.1**	**4.8**	**1.8**	**2.1**	**0.3**	**0.2**	**1.8**	**1.6**	**3.2**	**10.0**
Plasma												
Male	0 mg CPF/m³ (Control)	Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		S.D.	16.3	16.8	19.8	13.9	14.8	14.6	14.9	14.3	19.6	12.4
	13.3 mg CPF/m³ (Low) *	Mean	85.7	**51.3**	**36.7**	**34.0**	**35.3**	**37.5**	**48.5**	**50.9**	68.6	74.6
		S.D.	10.1	**3.9**	**4.6**	**6.1**	**6.2**	**6.6**	**6.7**	**5.1**	3.5	6.9
	66.7 mg CPF/m³ (High) *	Mean	**37.8**	**16.6**	**11.4**	**11.6**	**11.3**	**11.8**	**16.6**	**19.4**	**36.4**	**53.6**
		S.D.	**3.9**	**2.3**	**0.5**	**0.5**	**1.2**	**1.1**	**2.6**	**3.6**	**4.2**	**3.8**
Female	0 mg CPF/m³ (Control)	Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		S.D.	17.9	27.4	20.6	19.0	17.4	17.9	22.2	18.5	16.6	18.4
	13.3 mg CPF/m³ (Low) *	Mean	32.6	18.8	14.5	13.0	14.3	15.7	18.7	22.4	32.7	44.1
		S.D.	6.1	1.5	1.7	3.0	2.7	2.8	4.4	6.4	10.4	9.7
	66.7 mg CPF/m³ (High) *	Mean	18.9	10.1	7.3	7.4	7.4	6.8	8.5	8.6	10.1	10.1
		S.D.	3.4	1.6	0.4	1.4	0.7	0.2	1.4	2.3	1.6	0.9

* DOSE LEAST SQUARE MEAN STATISTICALLY DIFFERENT FROM CONTROL BY DUNNETT'S TEST, ALPHA=0.05.
BOLD VALUES (mean ± S.D.) = DOSE*TIME LEAST SQUARE MEAN STATISTICALLY DIFFERENT FROM CONTROL DOSE*TIME BY TUKEY'S TEST, ALPHA=0.05

Non-Guideline Acute Inhalation Study
With ChE and PK data

ACUTE INHALATION EXPOSURE OF ADULT CRL:CD(SD) RATS TO PARTICULATE CHLORPYRIFOS AEROSOLS: KINETICS OF CONCENTRATION-DEPENDENT CHOLINESTERASE (ChE) INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG

TABLE 5. Plasma, Lung, Red Blood Cell (RBC) and Brain Cholinesterase (ChE) Activity Summary Table – Phase 2

			Cholinesterase Activity (U/L)								
			During Exposure			After Exposure					
Tissue	Exposure		2	4	6	2	6	12	24	48	72
Plasma	0 mg CPF/m³ (Control)	Mean	2137.0	2416.3	2805.0	1905.2	2231.3	2498.0	2255.0	2904.8	2439.0
		Std. Dev.	814.5	1357.2	564.6	449.7	917.5	597.2	582.0	703.5	573.5
	3.7 mg CPF/m³ (Low)*	Mean	2047.0	1875.2	**1462.2 $**	1371.7	1662.7	1595.7	1878.2	1988.5	2063.8
		Std. Dev.	588.5	874.4	**577.1**	255.4	356.3	687.1	788.0	444.9	517.9
	12.9 mg CPF/m³ (Mid 1)*	Mean	1383.0	**768.8 $**	**648.7 $**	756.7	**848.7 $**	**940.5 $**	**884.3 $**	**1612.3 $**	1660.2
		Std. Dev.	671.3	**448.9**	**137.2**	374.2	**173.1**	**275.0**	**121.4**	**423.9**	487.0
	22.1 mg CPF/m³ (Mid 2)*	Mean	**841.3 $**	**722.3 $**	**718.7 $**	**624.2 $**	**641.7 $**	**1000.5 $**	1205.8	1765.0	2136.2
		Std. Dev.	**234.6**	**141.7**	**155.3**	**78.4**	**121.2**	**171.9**	299.7	745.2	584.5
	53.5 mg CPF/m³ (High)*	Mean	**810.7 $**	**760.5 $**	**864.0 $**	**409.5 $**	**656.5 $**	**853.8 $**	1107.7	**1486.3 $**	2201.3
		Std. Dev.	**178.2**	**222.4**	**309.2**	**70.6**	**226.0**	**468.6**	348.0	**359.1**	508.1
Lung	0 mg CPF/m³ (Control)	Mean	3361.5	3107.2	4453.2	3942.2	3070.7	2985.8	3002.2	3132.0	3087.0
		Std. Dev.	556.7	248.8	1013.6	956.4	310.0	662.6	452.5	426.0	308.7
	3.7 mg CPF/m³ (Low)*	Mean	2489.3	2431.0	**2347.3 $**	**2391.2 $**	2337.3	2399.7	3818.8	2920.0	2838.2
		Std. Dev.	280.6	528.0	**377.1**	**339.5**	469.7	448.3	1638.0	107.6	253.8
	12.9 mg CPF/m³ (Mid 1)*	Mean	2279.5	2258.2	**1263.8 $**	**1062.7 $**	**1440.8 $**	**1330.2 $**	2001.5	2266.5	2762.5
		Std. Dev.	292.9	971.5	**213.1**	**185.2**	**271.9**	**361.3**	833.5	258.5	543.4
	22.1 mg CPF/m³ (Mid 2)*	Mean	2718.8	**1470.2 $**	**1701.2 $**	**1688.3 $**	**1048.8 $**	2235.7	1684.8	2361.8	2462.7
		Std. Dev.	1048.4	**366.4**	**1258.9**	**848.2**	**384.3**	1672.9	229.3	336.8	356.3
	53.5 mg CPF/m³ (High)*	Mean	**1811.5 $**	**823.5 $**	**1123.8 $**	**782.3 $**	**650.5 $**	**1259.2 $**	1765.7	2157.7	2569.0
		Std. Dev.	**404.7**	**187.5**	**454.9**	**424.2**	**183.4**	**453.5**	509.4	387.9	668.5

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BOLD = $ DOSE*TIME LEAST SQUARE MEAN STATISTICALLY DIFFERENT FROM CONTROL DOSE*TIME BY TUKEY'S TEST, ALPHA=0.05

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ACUTE INHALATION EXPOSURE OF ADULT CRL:CD(SD) RATS TO PARTICULATE CHLORPYRIFOS AEROSOLS: KINETICS OF CONCENTRATION-DEPENDENT CHOLINESTERASE (ChE) INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG

Table 5. Plasma, Lung, Red Blood Cell (RBC) and Brain Cholinesterase (ChE) Activity Summary Table – Phase 2 (continued)

			Cholinesterase Activity (U/L)								
			During Exposure			After Exposure					
Tissue	Exposure		2	4	6	2	6	12	24	48	72
RBC	0 mg CPF/m³ (Control)	Mean	4942.3	3058.0	2763.7	3077.0	2346.0	4177.0	4824.3	4683.3	2987.3
		Std. Dev.	482.8	353.8	294.5	413.7	388.8	516.4	411.3	406.2	591.1
	3.7 mg CPF/m³ (Low)	Mean	4880.7	3285.7	**3795.7 $**	**4433.3 $**	2388.7	3960.0	**3410.7 $**	**3140.0 $**	2810.7
		Std. Dev.	312.7	231.1	**812.3**	**217.1**	335.7	517.9	**202.5**	**550.7**	441.6
	12.9 mg CPF/m³ (Mid 1)*	Mean	**2365.0 $**	3269.3	**1651.3 $**	**1547.7 $**	1654.0	**1053.3 $**	**1003.7 $**	**1668.0 $**	**1347.7 $**
		Std. Dev.	**516.1**	732.4	**408.0**	**276.6**	284.7	**315.4**	**191.6**	**397.4**	**324.8**
	22.1 mg CPF/m³ (Mid 2)*	Mean	**3830.0 $**	3137.3	**865.0 $**	**964.0 $**	**935.7 $**	**1788.0 $**	**2233.7 $**	**2285.7 $**	**1675.3 $**
		Std. Dev.	**428.8**	644.3	**692.3**	**437.7**	**198.4**	**525.2**	**480.7**	**393.5**	**362.5**
	53.5 mg CPF/m³ (High)*	Mean	**3084.0 $**	**1041.3 $**	**697.3 $**	**136.0 $**	**196.0 $**	**728.3 $**	**1338.3 $**	**2193.0 $**	2497.3
		Std. Dev.	**520.6**	**411.6**	**506.0**	**107.5**	**138.0**	**422.8**	**143.1**	**360.3**	512.8
Brain	0 mg CPF/m³ (Control)	Mean	51934.0	51251.0	46603.5	51213.3	52866.7	49703.3	51035.3	50707.3	51079.7
		Std. Dev.	1801.2	2181.0	7672.8	3033.2	4121.0	3174.8	9757.8	1553.0	3195.6
	3.7 mg CPF/m³ (Low)	Mean	60444.5	51387.2	48869.7	50240.7	50865.0	50402.8	59303.5	52368.7	49913.2
		Std. Dev.	15821.4	2007.3	4146.8	3829.0	978.5	2594.6	8738.1	2412.9	2500.4
	12.9 mg CPF/m³ (Mid 1)	Mean	50517.8	51131.5	53959.0	45971.0	43049.8	42568.5	43481.2	46646.0	45820.5
		Std. Dev.	2926.0	5768.1	17785.8	11542.4	7041.6	6773.1	2540.8	2791.4	3202.0
	22.1 mg CPF/m³ (Mid 2)	Mean	50802.7	45085.0	39618.3	41852.3	41749.0	49708.0	62720.4	49260.8	42920.8
		Std. Dev.	6248.3	5222.1	7090.6	5902.0	5357.8	18035.1	28574.0	7240.0	4618.1
	53.5 mg CPF/m³ (High)*	Mean	45559.2	47809.3	47151.0	39872.7	42302.3	45273.7	42248.2	43136.2	38914.0
		Std. Dev.	3611.3	8874.2	13701.6	10216.5	15338.1	14986.3	6739.1	7169.2	3736.7

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ACUTE INHALATION EXPOSURE OF ADULT CRL:CD(SD) RATS TO PARTICULATE CHLORPYRIFOS AEROSOLS: KINETICS OF CONCENTRATION-DEPENDENT CHOLINESTERASE (ChE) INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG

Table 6. Normalized Plasma, Lung, Red Blood Cell (RBC) and Brain Cholinesterase (ChE) Activity Summary Table – Phase 2 (continued)

			Normalized Cholinesterase Activity (% of Control Values)								
			During Exposure			After Exposure					
Tissue	Exposure		2	4	6	2	6	12	24	48	72
RBC	0 mg CPF/m^3 (Control)	Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		Std. Dev.	9.8	11.6	10.7	13.4	16.6	12.4	8.5	8.7	19.8
	3.7 mg CPF/m^3 (Low)	Mean	98.8	107.4	**137.3**	**144.1**	101.8	94.8	**70.7**	**67.0**	94.1
		Std. Dev.	6.3	7.6	**29.4**	**7.1**	14.3	12.4	**4.2**	**11.8**	14.8
	12.9 mg CPF/m^3 (Mid 1)*	Mean	**47.9**	106.9	**59.8**	**50.3**	70.5	**25.2**	**20.8**	**35.6**	**45.1**
		Std. Dev.	**10.4**	24.0	**14.8**	**9.0**	12.1	**7.6**	**4.0**	**8.5**	**10.9**
	22.1 mg CPF/m^3 (Mid 2)*	Mean	**77.5**	102.6	**31.3**	**31.3**	**39.9**	**42.8**	**46.3**	**48.8**	**56.1**
		Std. Dev.	**8.7**	21.1	**25.1**	**14.2**	**8.5**	**12.6**	**10.0**	**8.4**	**12.1**
	53.5 mg CPF/m^3 (High)*	Mean	**62.4**	**34.1**	**25.2**	**4.4**	**8.4**	**17.4**	**27.7**	**46.8**	83.6
		Std. Dev.	**10.5**	**13.5**	**18.3**	**3.5**	**5.9**	**10.1**	**3.0**	**7.7**	17.2
Brain	0 mg CPF/m^3 (Control)	Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		Std. Dev.	3.5	4.3	16.5	5.9	7.8	6.4	19.1	3.1	6.3
	3.7 mg CPF/m^3 (Low)	Mean	116.4	100.3	104.9	98.1	96.2	101.4	116.2	103.3	97.7
		Std. Dev.	30.5	3.9	8.9	7.5	1.9	5.2	17.1	4.8	4.9
	12.9 mg CPF/m^3 (Mid 1)	Mean	97.3	99.8	115.8	89.8	81.4	85.6	85.2	92.0	89.7
		Std. Dev.	5.6	11.3	38.2	22.5	13.3	13.6	5.0	5.5	6.3
	22.1 mg CPF/m^3 (Mid 2)	Mean	97.8	88.0	85.0	81.7	79.0	100.0	122.9	97.1	84.0
		Std. Dev.	12.0	10.2	15.2	11.5	10.1	36.3	56.0	14.3	9.0
	53.5 mg CPF/m^3 (High)*	Mean	87.7	93.3	101.2	77.9	80.0	91.1	82.8	85.1	76.2
		Std. Dev.	7.0	17.3	29.4	19.9	29.0	30.2	13.2	14.1	7.3

* DOSE LEAST SQUARE MEAN STATISTICALLY DIFFERENT FROM CONTROL BY DUNNETT'S TEST, ALPHA=0.05.
BOLD VALUES (mean ± S.D.) = DOSE*TIME LEAST SQUARE MEAN STATISTICALLY DIFFERENT FROM CONTROL DOSE*TIME BY TUKEY'S TEST, ALPHA=0.05

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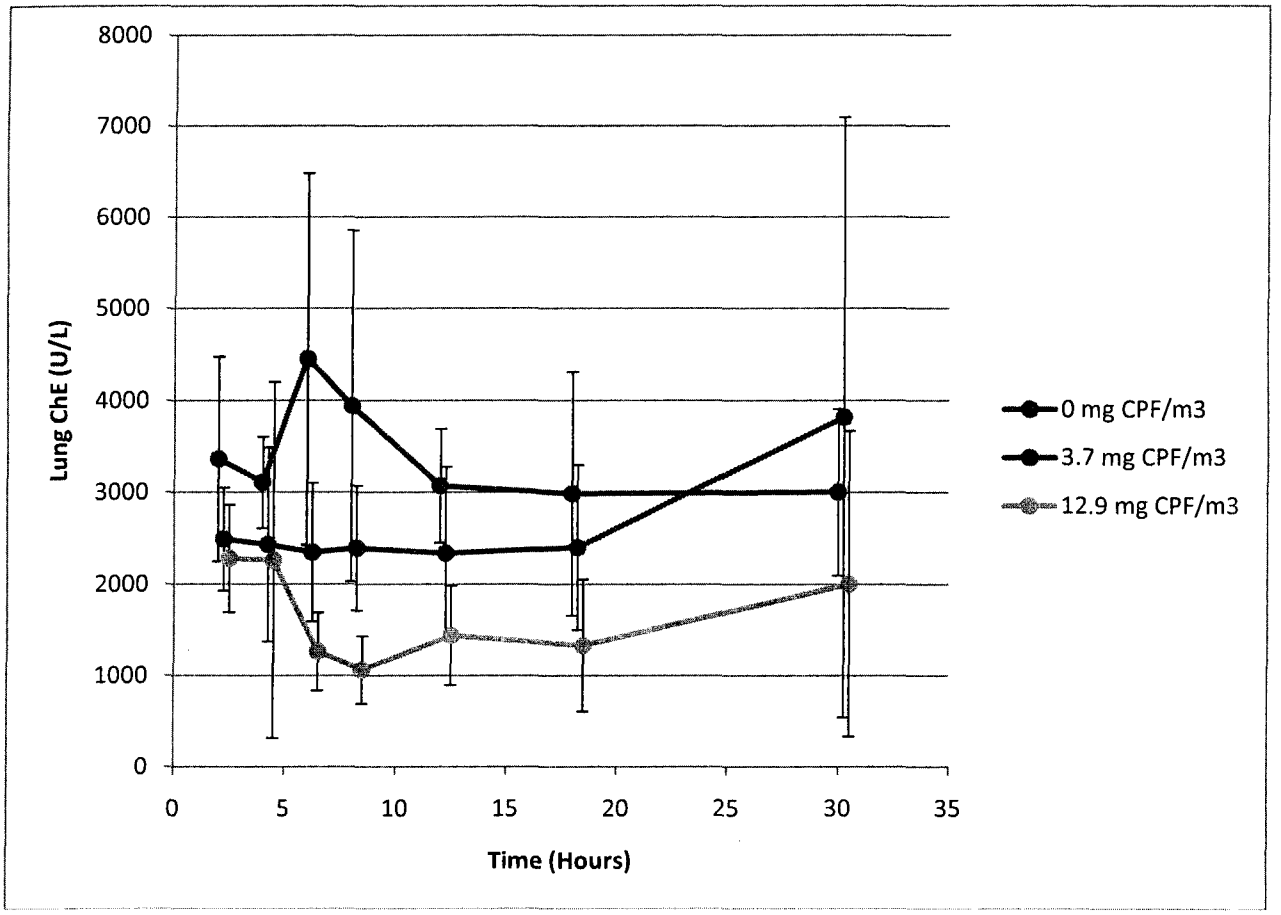
ACUTE INHALATION EXPOSURE OF ADULT CRL:CD(SD) RATS TO PARTICULATE CHLORPYRIFOS AEROSOLS: KINETICS OF CONCENTRATION-DEPENDENT CHOLINESTERASE (ChE) INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG

TABLE 7. Blood Concentrations (ng/g) of Chlorpyrifos (CPF), Chlorpyrifos-oxon (OXON), and 3,5,6-trichloro-2-pyridinol (TCP) at selected sample times during and after exposure to 3.7, 12.9, 22.1, or 53.5 mg/m^3 particulate CPF – Phase 2

Sample Times (hours)		Chlorpyrifos Exposure Concentration											
		3.7 mg/m^3			12.9 mg/m^3			22.1 mg/m^3			53.5 mg/m^3		
		CPF	OXON	TCP	CPF	OXON	TCP	CPF	OXON	TCP	CPF	OXON	TCP
Exposure	2	2.6 (0.2)	<LLQ	<LLQ	8.3 (3.1)	0.18 (n=1)	<LLQ	16.3 (4.2)	<LLQ	<LLQ	54.9 (7.7)	0.22 (0.10)	24.4 (15.3)
	4	4.3 (0.5)	<LLQ	<LLQ	31.9 (9.6)	0.12 (n=1)	<LLQ	33.9 (10.0)	0.14 (0.03)	<LLQ	64.8 (29.2)	0.21 (0.12)	33.6 (10.6)
	6	5.7 (2.4)	<LLQ	<LLQ	34.7 (5.0)	<LLQ	71.3 (30.2)	28.7 (7.5)	0.20 (0.03)	60.3 (37.4)	59.1 (22.5)	0.13 (0.02)	106.2 (12.4)
Post-Exposure	2	0.3 (0.1)	<LLQ	32.8 (15.6)	5.2 (2.1)	<LLQ	214.2 (60.5)	4.5 (0.8)	<LLQ	99.4 (37.4)	18.5 (5.0)	0.12 (n=1)	520.7 (246.5)
	6	<LLQ	<LLQ	109.2 (37.3)	0.8 (0.1)	<LLQ	474.1 (399.2)	1.1 (0.2)	<LLQ	510.0 (260.7)	2.8 (0.6)	<LLQ	1150.5 (174.1)
	12	<LLQ	<LLQ	213.6 (41.0)	0.5 (0.1)	<LLQ	674.3 (85.4)	0.2 (0.1)	<LLQ	897.1 (222.9)	1.5 (0.4)	<LLQ	2439.4 (589.6)
	24	<LLQ	<LLQ	301.3 (110.6)	0.3 (0.1)	<LLQ	1110.1 (103.2)	0.1 (0.1)	<LLQ	1166.4 (585.3)	0.4 (0.1)	<LLQ	1798.0 (849.3)
	48	0.14 (0.01)	<LLQ	190.9 (58.6)	<LLQ	<LLQ	823.5 (283.2)	<LLQ	<LLQ	701.1 (91.5)	0.1 (n=1)	<LLQ	1983.2 (519.7)
	72	0.25 (0.03)	<LLQ	93.3 (25.5)	<LLQ	<LLQ	211.3 (89.0)	<LLQ	<LLQ	405.4 (91.7)	<LLQ	<LLQ	846.8 (230.7)

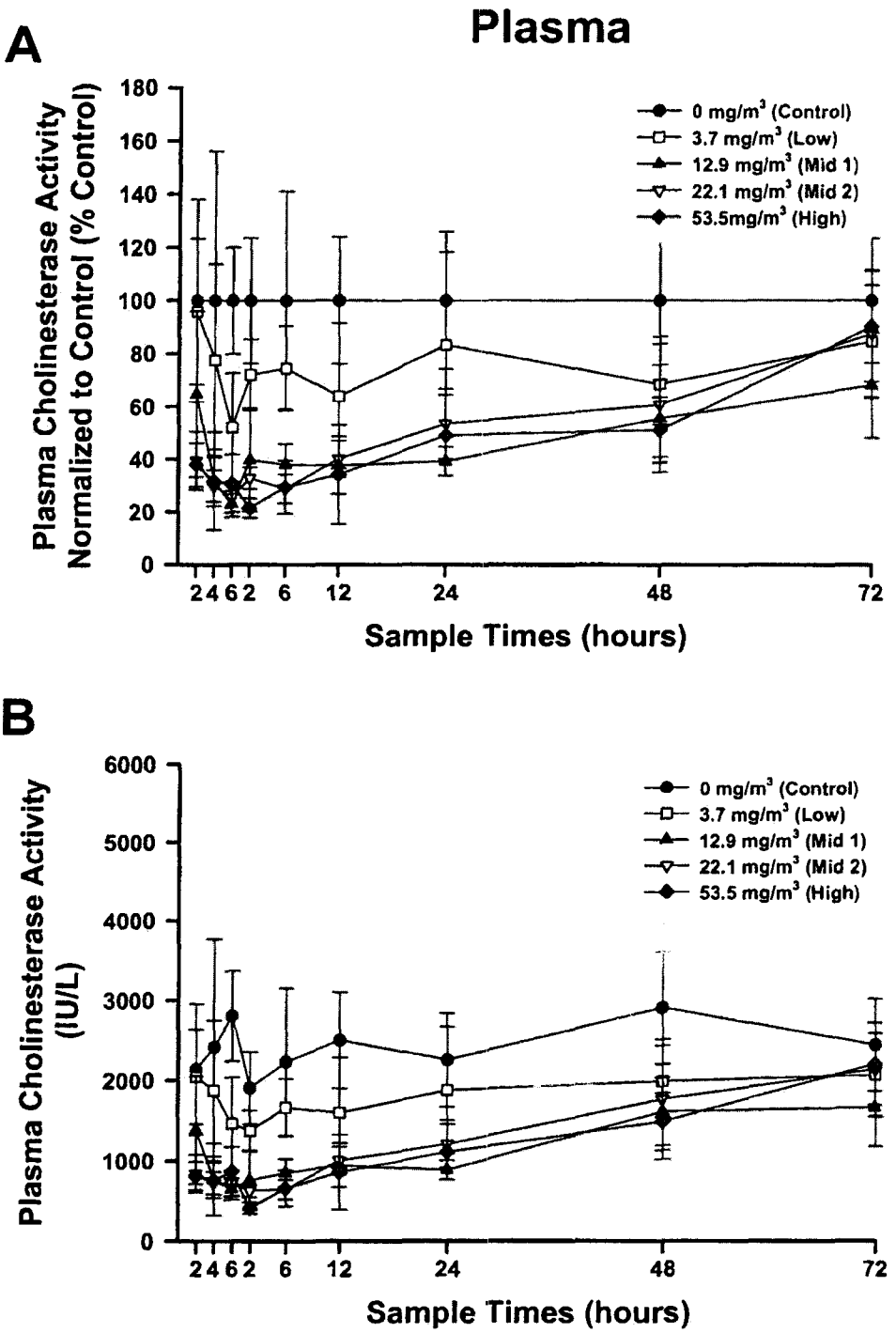
Values represent group Mean and (Std. Dev); (n=1) reported if only 1 value ≥ LLQ
LLQ (CPF and OXON) = 0.1 ng/g; *LLQ* (TCP) = 10.2 ng/g

FIGURE 1. LUNG ChE INHIBITION.



ACUTE INHALATION EXPOSURE OF ADULT CRL:CD(SD) RATS TO PARTICULATE CHLORPYRIFOS AEROSOLS: KINETICS OF CONCENTRATION-DEPENDENT CHOLINESTERASE (ChE) INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG

FIGURE 4. Effect of Inhaled Particulate CPF on Plasma Cholinesterase Activity – Phase 2



EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** 3/31/2011
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** Myron Ottley
Risk Assessment Branch III, Health Effects Division (7509P) **Date:** 3/31/11
Template version 02/06

TXR#: 0055409

DATA EVALUATION RECORD

STUDY TYPE: 4-Week Dietary Immunotoxicity Study – Rat
OPPTS 870.7800

PC CODE: 059101 **DP BARCODE:** D380055

TEST MATERIAL (PURITY): Chlorpyrifos Technical (99.8% a.i.)

SYNONYMS: O,O-Diethyl O-(3,5,6-trichloro-2-pyridinyl)ester phosphorothioic acid; chlorpyrifos-ethyl; clorpyrifos, clorpyriphos

CITATION: Boverhof, D., J. Murray, and R. Sura (2010) Chlorpyrifos: assessment of immunotoxic potential using the sheep red blood cell assay after 28-day dietary exposure to rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company (Midland, MI). Laboratory Project Study ID: 101023, June 28, 2010. MRID 48139304. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268.

EXECUTIVE SUMMARY:

In an immunotoxicity study (MRID 48139304), Chlorpyrifos Technical (99.8% a.i., Lot No. KC28161419) was administered in the diet to 10 female Crl:CD(SD) rats/dose at nominal dose levels of 0, 0.4, 2, or 10 mg/kg/day (actual dose levels of 0, 0.416, 2.13, or 10.7 mg/kg/day) for 28 days. The female rat was determined to be the appropriate test species/sex for this study. Cyclophosphamide in sterile saline was intraperitoneally administered to the positive control group on Days 24 to 28 at a rate of 20 mg/kg body weight/day. On Day 24, all animals received intravenous injection of 0.5 mL sheep red blood cells (SRBCs) in isotonic saline (2 x 10^8 SRBCs/mL). T-cell dependent antibody response (TDAR) was evaluated at Day 29.

There were no statistically significant effects of treatment with chlorpyrifos on mean body weights, body weight gains, food consumption, clinical signs, gross anatomy, or hematological parameters. No unscheduled mortalities occurred in any study group. Statistically significant decreases in mean red blood cell (RBC) cholinesterase (ChE) activity were seen in all test substance treatment groups. Mean brain ChE activity was significantly decreased in the mid- and high-dose groups. In the positive control group, mean body weights and body weight gains were lower than the control value throughout the study. For systemic toxicity related to treatment with chlorpyrifos, the NOAEL is 10 mg/kg/day (the highest dose tested) based on no effects were seen in clinical observations, body weight, food consumption, and hematological

DATA EVALUATION RECORD

Chlorpyrifos
PC Code: 059101
TXR# 0055409
MRID# 481393-01

4-Week Dietary Immunotoxicity Study in the Rat
OPPTS 870.7800

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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William Burnett, [illegible]

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Kristin Lagan, Ph.D.

Quality Control _____ Date 9-13-10
[illegible]

Contract Number: EP [illegible]
Work Assignment No.: [illegible]

Task No.: [illegible]
EPA WAM: M. Cortney Dunerty

This review may be altered by EPA subsequent to the contractors' signatures above.

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parameters. The LOAEL for systemic toxicity was not established. For neurotoxic effects, the LOAEL is 0.4 mg/kg/day (the lowest dose tested), based on decreased RBC cholinesterase activity. The NOAEL for neurotoxic effects was not established.

For immunotoxicity, there were no treatment-related effects on mean absolute and relative spleen and thymus weights or hematological parameters at any dose level. The anti-SRBC IgM titers did not show statistically significant differences among the treatment and control groups. Anti-SRBC IgM titers for the 2 and 10 mg/kg/day treatment groups were decreased (64% and 41%, respectively) compared with the control. However, the decreased response in these dose groups may have been due, in part, to a high mean value for the control group. The control value was out of the historical control ranges. The biological significance of these observations also was confounded by the lack of a clear dose response (the decrease was greater for the mid-dose group than for the high-dose group). The distribution of individual animal data did not demonstrate any trend or suppression among treatment and control groups. There was no evidence demonstrated a significant suppression of the anti-SRBC IgM response in animals treated with chlorpyrifos. The positive control data had demonstrated the validity of the assay.

The NK cell activity was not evaluated in this study. Toxicity database of repeat-dose studies (2-week, 28-day, 90-day, 2-year) studies in rats and mice showed no treatment-related effects on spleen and thymus weights and histopathology parameters that would suggest the potential for immunotoxicity. Under the HED guidance, if the TDAR assay is negative and evaluation of observational endpoints from all available toxicology database provide no evidence of immunotoxicity, the test article is considered negative for immunotoxicity and evaluation of NK activity is not necessary.

Under conditions of this study, the NOAEL for immunotoxicity is 10 mg/kg/day (the highest dose tested) based on the overall weight-of-evidence. A lack of dose-related response for anti-SRBC IgM titers at the mid- and high-dose levels, a lack of statistical significance at any dose level, and a lack of evidence of other immunological effects (absolute and relative spleen and thymus weights, hematological parameters). A LOAEL for immunotoxicity was not established.

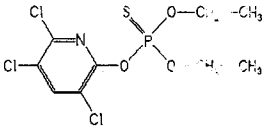
This immunotoxicity study in the rat is considered as **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. There were no claims of data confidentiality. The study was conducted in accordance with USEPA FIFRA GLPs Title 40 CFR, Part 160, as well as with The Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), OECD, and EC GLPs.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Chlorpyrifos, Technical

Description:	Not provided
Lot/batch #:	Lot # KC28161419, Reference No. TSN101285 (Dow AgroSciences LLC, Indianapolis IN)
Purity:	99.8% a.i.
Compound stability:	Not provided
CAS # of TGAI:	2921-88-2
Structure:	

2. Vehicle and/or positive control: The vehicle for the test substance was the diet (see details below). Cyclophosphamide monohydrate (Sigma-Aldrich, Saint Louis MO, Lot # 079K1569, 100.5 % purity as per non-GLP certificate of analysis) was used as the positive control.

3. Test animals:

Species:	Rat (female, nulliparous and nonpregnant)
Strain:	Crl:CD(SD)
Age/weight at study initiation:	approximately 7 weeks; 149.1 to 184.9 grams
Source:	Charles River Laboratories (CRL), Inc., Portage, MI
Housing:	Stainless steel cages with wire mesh floors, suspended above absorbent paper; non-woven gauze was placed in the bottom of the cages to cushion the rodent feet from the flooring; one animal/cage after study group assignment
Diet:	LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, MO) in meal form provided in feed crocks, *ad libitum*; food was analyzed by the supplier to confirm adequate nutrition and to quantify the concentration of selected contaminants
Water:	Public drinking water supplied by a pressure activated lixit valve-type watering system, *ad libitum*; water is periodically analyzed for chemical/biological contamination by the municipal water department; specific chemical contaminant analyses conducted at periodic intervals by an independent testing facility
Environmental conditions:	**Temperature:** 22EC with a tolerance of ±1EC; maximum permissible excursion of ±3EC **Humidity:** 40-70% **Air changes:** 12-15/hour **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	Minimum of one week prior to start of study; 2-3 animals/stainless steel cages in rooms designed to maintain adequate temperature, humidity and photocycle

B. STUDY DESIGN:

1. **In life dates:** Start: February 23, 2010; End: March 23, 2010

2. **Selection of test species and gender**: The female rat was determined to be the appropriate species and sex for chlorpyrifos. Previous studies with this chemical have indicated that rats are more sensitive than mice and females are more sensitive than males based on cholinesterase (ChE) inhibition (most sensitive effect in all animal species evaluated).

3. **Animal assignment**: Animals were stratified by body weight and then randomly assigned to test groups using a computer program designed to increase the probability of

uniform group mean weights and standard deviations at the start of the study. Animals were assigned to the test groups noted in Table 1. The test substance dose groups are nominal doses based on diet concentrations, and body weight and food consumption data. On Days 24-28, animals in the positive control group were administered cyclophosphamide in isotonic sterile saline (20 mg/mL) by intraperitoneal injection at a dose volume of 1 mL/kg body weight to achieve a dose of 20 mg/kg.

TABLE 1 Study design

Test group	Dose level of chlorpyrifos (mg/kg/day) [a]	# Animals (female)
Control [b]	0	10
Low Dose	0.4	10
Mid Dose	2.0	10
High Dose	10	10
Positive control [c]	0	10

[a] Data were obtained from page 15 (Text Table 1) of the study report.
[b] Vehicle control (referred to in this review as the "control")
[c] Dosed with 20 mg/kg cyclophosphamide by intraperitoneal injection on Days 24-28.

4. **Dose selection rationale:** In previous repeated dose studies, inhibition of plasma ChE has been observed at doses near 1 mg/kg/day while inhibition of brain ChE occurred in the range of 5-15 mg/kg/day. For this study, the high-dose of 10 mg/kg/day was selected to produce some measurable sign of general toxicity and/or inhibition of brain cholinesterase without producing significant stress, malnutrition, or mortalities. The lower dose levels were expected to provide dose-response data for any treatment-related effects seen in the high-dose group. The low dose was expected to be a no-observed-effect level. The dose of cyclophosphamide was chosen because it was expected to result in an approximately 90% reduction in the anti-SRBC antibody response.[1]

5. **Diet preparation and analysis:** A premix was prepared prior to the start of the study by dissolving the test substance in acetone and adding the solution to rodent chow. Diets were prepared weekly based on the most recent body weight and food consumption data. The initial concentrations of the test substance in each diet were calculated based on historical body weights and food consumption data. Diets were prepared by serially diluting the premix with ground food. The control diets contained food prepared using an amount of acetone equivalent to the amount in the premix that was used to prepare the high-dose diet. The premix and ground food were mixed and left overnight in a vented area to volatilize the acetone. The diet concentrations were not adjusted for purity.

Homogeneity and concentration control analyses were performed pre-exposure. Three samples were analyzed for the premix, two each for the control and the 2 mg/kg/day treatment groups; and 6 each for the 0.4 and 10 mg/kg/day treatment groups. The homogeneity of the premix, and the 0.4 and 10 mg/kg/day treatment groups was determined

[1] References cited (not available to the reviewer): 1) Woolhiser, M. and M. Holsapple (2002) Comparison of antibody forming cell and IGM responses to SRBC antigen in CD rats following cyclophosphamide administration. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan; and 2) Loveless, S., G. Ladics, C. Smith, et al. (2002) Interlaboratory study of the primary antibody response to sheep red blood cells in outbred rodents. The Toxicologist 66 (1-S), page 1164.

along with dose confirmation. Test substance concentrations in the diet were analyzed using a solvent extraction method (solvent not specified) followed by analysis with gas chromatography-mass spectrometry (GC/MS). A stability analysis was not performed as part of the present study; according to the authors, the stability of chlorpyrifos in rodent diet for at least 30 days has previously been demonstrated at concentrations including those used in the present study.[2]

Results:

Concentration analysis: The mean concentration in the samples from the premix and the diet of each of the test substance treatment groups ranged from 97.7% to 111.1 % of the targeted concentration. Mean measured (targeted) diet concentrations in w/w% were 0.204 (0.200), 0.000485 (0.000436), 0.00228 (0.00218), and 0.0107 (0.0109) for the premix and the 0.4, 2, and 10 mg/kg/day dose level groups, respectively (data are from page 39, Table 3 of the study report). Chlorpyrifos was not detected (was below the limit of quantitation) in the control diet.

Homogeneity analysis: The investigators conclude that the test substance was distributed homogeneously in the diet for all test groups, based on the relative standard deviations for the samples from the premix, 0.4, and 10 mg/kg/day test groups (3.1, 6.4 and 5.3%, respectively) (data are from page 39, Table 3 of the study report). It was not indicated in the report from where in the dietary mixture the samples were collected, either relative to the container or to each other.

Stability analysis: A stability analysis was not conducted in conjunction with the present study. Based on previous studies (see footnote 2) chlorpyrifos was found to be stable

6. **Immunization:** On Day 24 animals in all groups received a single dose (0.5 mL intravenous injection) of sheep red blood cells (SRBCs) in sterile solution (2×10^8 SRBCs/mL) via the lateral tail vein to assess the T-cell dependent antibody response of the test animals.

7. **Statistics:** Body weights, food consumption, organ weights, appropriate hematologic data, and the results of the SRBC ELISA were evaluated by Bartlett's test for equality of variances ($p \leq 0.01$). Depending on the results of Bartlett's test, additional data analysis was performed using a parametric (Steel and Torrie) or nonparametric (Hollander and Wolfe) analysis of variance (ANOVA). If significant at $p \leq 0.05$, the ANOVA was followed by Dunnett's test ($p \leq 0.05$) or the Wilcoxon Rank-Sum test ($p \leq 0.05$) with a Bonferroni correction for multiple comparisons to the control. For the ELISA data, the positive control data were compared to the vehicle control data in a separate analysis from the treated groups and vehicle control. Detailed clinical observation (DCO)

[2] References cited (not available to the reviewer): 1) Maurissen, J., M. Dryzga, T. Card, et al. (2004) Dietary exposure to chlorpyrifos: effects on butylcholinesterase in the rat. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan; 2) Brzak, K. (1991a) Stability data. Analytical code 89-361. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland Michigan; 3) Brzak, K. (1991b) Stability data. Analytical code 89-174. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan; and 4) Szabo, J., J. Young, and M. Grandjean (1988). Chlorpyrifos: 13 week dietary toxicity study in Fischer-344 rats. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

incidence data for scored observations were statistically analyzed by a z-test of proportions comparing each treated group to the control ($p \leq 0.05$). Statistical outliers were identified by a sequential test ($p \leq 0.02$), but routinely excluded only from food consumption statistics; according to the investigators, outliers may have been excluded from other analyses for documented, scientifically sound reasons.

The Reviewer considers the analyses used to be appropriate. It is to be noted that the means for body weight gains, red blood cell (RBC) indices, and white blood cell (WBC) counts were not statistically compared with the respective control values.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were checked at least once a day at approximately the same time, usually the morning, for general health and significant clinical abnormalities. The abnormalities checked for included, but were not limited to: decreased/increased activity, repetitive behavior, vocalization, incoordination/limping, injury, neuromuscular function (convulsions, fasciculations, tremors, twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), alterations in fecal consistency, and fecal/urinary quantity. Animals were checked for moribundity and mortality, and the availability of food and water at least twice daily.

1b. Clinical examinations: Detailed clinical observations were made on animals in the control and test substance treatment groups prior to test substance administration and at weekly intervals during the treatment period at approximately the same time each day. The examination included cage-side, hand-held, and open-field observations. The observations were recorded categorically (i.e., descriptively) or were ranked (rated numerically using defined scales). Detailed lists of the specific observations that were made and of the ranking system were provided (page 38, Table 2 and pages 143-146, Appendix A of the study report).

2. **Body weight:** Body weights were measured for the control and the test substance treatment groups before commencement of test substance administration (Day –4), twice weekly during administration (Days 1, 4, 8, 11, 15, 18, 22, 25) and on the day of sacrifice (terminal body weight, Day 29). Body weights for the positive control group were measured once weekly during the treatment period (Days 1, 8, 15, 22) and on the day of sacrifice (Day 29). Body weight gains were calculated relative to the respective weights on Day 1.

3. **Food consumption and compound intake:** Food consumption was determined for the control and the test substance treatment groups pre-exposure (Day -4 to Day 1), twice during the first week (Days 1-4, and 4-8) and weekly thereafter (Days 8-15, 15-22, and 22-29). Food consumption (grams) was determined by weighing the food containers at the beginning and end of a measurement interval. The food consumed (grams/day) was calculated for each animal as the difference in the weight of the food container divided by the number of days in the measurement interval.

The test material intake (TMI), as mg/kg/day, was calculated using concentrations of test

substance in the diet, body weights, and food consumption data as follows:

$$TMI = \frac{\text{food consumption (g/day) x \% of test material in food/100 x 1000 mg/g}}{\text{[current body wt. (grams) + previous body wt (grams)]/2 divided by 1000 g/kg}}$$

4. Sacrifice and pathology: At the scheduled necropsy on Day 29, animals were anesthetized by inhalation with a mixture of isoflurane vapors and medical grade oxygen. The animals were weighed and blood was taken from the orbital sinus for determination of anti-SRBC IgM, and for assessment of hematology parameters and levels of cholinesterase (ChE) activity in red blood cells. Animals were not fasted before blood collection. Animals were sacrificed under anesthesia; their tracheas were exposed and clamped and the animals were killed by decapitation.

4a. Anatomic pathology:

Gross necropsy: A limited gross necropsy, including an examination of external tissues and all orifices, was performed on all animals. Eyes were examined *in situ* by applying a moistened microscope slide to each cornea. The head was removed from all animals. The brain was removed from the animals in the control and test substance treatment groups, dissected into right and left hemispheres and the right hemisphere weighed. Both hemispheres were quick frozen in liquid nitrogen and stored at -80 °C until analysis for ChE activity. The skin from all animals was reflected from the carcass; the thoracic and abdominal cavities were opened and the viscera examined. All viscera were removed and re-examined, and selected tissues were incised.

Organ weights: Spleens and thymuses from all study animals were trimmed and weighed immediately and the ratios of organ weight to terminal body weight were calculated.

Organ fixation: For the control and test substance treatment groups, samples of the stomach, liver, spleen, thymus, sternum, mesenteric lymph node, Peyer's patch (GALT), and gross lesions were preserved in neutral, phosphate-buffered 10% formalin.

4b. Clinical pathology:

Hematology: Hematological parameters were examined for the control and test substance treatment groups. Blood samples used for a complete blood count were mixed with EDTA. Blood smears were stained with Wright-Giemsa stain and archived in case future evaluation was warranted. Hematologic parameters were assayed using the Bayer Advia 120 Hematology Analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY). The parameters assayed were: hematocrit, hemoglobin concentration, RBC count, total white blood cell (WBC) count, differential WBC count, platelet count, and reticulocyte count. The following indices were determined: mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration.

Red blood cell (RBC) cholinesterase activity: According to the investigators, the inhibition of cholinesterase (ChE) is the most sensitive effect of chlorpyrifos in all evaluated species, regardless of exposure duration, with rats being more sensitive than mice. RBC ChE activity was measured for animals in the control and test substance treatment groups. Blood samples

for RBC ChE analyses were placed on ice immediately after collection, then centrifuged to separate the plasma and RBCs. The isolated RBCs were diluted 1:20 in 1% Triton X-100 solution buffered to pH 8 with Na_2HPO_4, separated into two aliquots, frozen, and stored at -80 °C until analyzed. One aliquot of each sample was shipped on dry ice to a contract research laboratory (WIL Research Laboratories, LLC, Ashland, OH) for ChE analysis. The other RBC aliquot and all plasma samples were frozen, and stored at -80EC in case further analyses were needed.

Brain cholinesterase activity: Brain ChE activity was measured for animals in the control and test substance treatment groups. The right brain hemispheres, collected during the gross necropsy and stored frozen at -80 °C, were shipped on dry ice to a contract research laboratory (WIL Research Laboratories, LLC, Ashland, OH) for ChE analysis. The left brain hemispheres were stored at Toxicology &Environmental Research and Consulting in case future analyses were warranted.

5. **Immunotoxicity**:

a. **Anti-SRBC IgM concentration- ELISA**: The immune response was evaluated using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Life Diagnostics, West Chester, PA). The ELISA was performed according to the manufacturer's recommendations with some modifications. The assay used detergent solubilized SRBC ghosts for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rat IgM antibodies for detection. Blood samples designated for the ELISA assay were collected in serum separator tubes, the blood allowed to clot, the serum separated from the blood cells by centrifugation, and stored at -20 °C until the assay was conducted. Serum samples were diluted and incubated in the microtiter wells for 45 minutes; the wells were then washed and the HRP conjugate added and incubated for 45 minutes. The wells were washed to remove unbound HRP-labeled antibodies, and tetramethylbenzidine peroxidase reagent added and incubated for 20 minutes at ambient temperature. Color (blue) development was stopped by addition of Stop Solution, which changed the color to yellow. The optical density was measured spectrophotometrically at 450 nm; the concentration of anti-SRBC IgM was proportional to the optical density and expressed as units (u)/mL. The concentration of the anti-SRBC IgM was determined with a standard curve prepared by serial dilution of lyophilized rat anti-SRBC standard stock provided by the manufacturer.

b. **Natural Killer (NK) cell activity assay**: No NK cell activity was evaluated.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no treatment-related observations for the ranked parameters. Dermatitis was observed in one animal in the 0.4 mg/kg/day group on Days 22-28, and in 2 animals in the 10 mg/kg/day treatment group on Day 15 and from Days 15-28, respectively (data are from pages 65 to 69, Appendix Table 2). The cases of dermatitis were considered spurious and not treatment-related because of the isolated incidences and lack of dose-response.

2. **Mortality:** No unscheduled mortalities occurred during the course of the study.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** There were no statistically significant differences between the mean body weights of the test substance treatment and positive control groups and those of the vehicle control; the means of the body weight gains were not statistically compared with the control. Mean body weights and body weight gains in the 0.4 and 2 mg/kg/day treatment groups were considered by the investigators to be comparable to the control, while those of the 10 mg/kg/day treatment group were described as being slightly lower than the control through Day 22. The observations in the high-dose groups were considered to have limited biological significance because of the minimal nature of the difference and the lack of a dose response, and because the mean body weight and body weight gains were comparable to the controls by Day 29. On Day 25, the control and 0.4 and 2 mg/kg/day groups showed a decrease in mean body weight and body weight gain; these observations were attributed to the stress of the SRBC injections on Day 24. Mean body weights and body weight gains of the positive control group were described as slightly lower than the control throughout the study; these differences were considered by the investigators to be due to normal body weight variability. Mean body weights and mean body weight gains for all study groups during the treatment period are presented in Tables 2 and 3, respectively.

TABLE 2 Average body weight during the treatment period [a]

Treatment group (mg/kg/day chlorpyrifos)	Mean body weight (grams±SD)								
	Day 1	Day 4	Day 8	Day 11	Day 15	Day 18	Day 22	Day 25	Day 29
Females (n=10/group)									
0 (Control)	172.0 ±7.7	184.2 ±10.5	197.8 ±13.3	201.2 ±15.7	214.0 ±17.1	216.5 ±17.3	226.6 ±17.6	223.7 ±17.5	229.4 ±20.2
0.4	172.2 ±8.3	184.5 ±8.4	196.9 ±10.3	202.1 ±9.4	212.5 ±11.3	215.7 ±10.2	226.4 ±9.2	223.9 ±9.5	229.9 ±11.3
2	171.6 ±8.7	183.3 ±9.2	197.3 ±11.4	200.7 ±9.4	211.8 ±11.2	213.8 ±12.8	225.1 ±14.7	222.5 ±14.8	232.7 ±15.0
10	173.0 ±8.2	181.4 ±10.7	193.3 ±12.9	198.6 ±11.5	207.1 ±14.9	213.3 ±18.8	223.7 ±24.0	223.9 ±25.9	223.5 ±26.0
Positive control – cyclophosphamide	168.9 ±8.8	-	188.7 ±14.4	-	202.7 ±13.9	-	222.1 ±18.8	-	219.3 ±19.5

[a] Data were obtained from pages 43 to 45 (Table 6) of the study report.

TABLE 3 Average body weight gain during the treatment period [a]

Treatment group (mg/kg/day chlorpyrifos)	Mean body weight gain (grams±SD)							
	Day 4	Day 8	Day 11	Day 15	Day 18	Day 22	Day 25	Day 29
Females (n=10/group)								
0 (Control)	12.2 ±3.9	25.8 ±7.5	29.3 ±10.0	42.1 ±11.3	44.6 ±10.8	54.6 ±11.1	51.7 ±11.3	57.5 ±13.1
0.4	12.3 ±4.4	24.7 ±7.1	29.8 ±7.1	40.3 ±8.3	43.5 ±7.7	54.1 ±8.3	51.7 ±6.0	57.7 ±6.4
2	11.6 ±4.4	25.7 ±8.2	29.1 ±6.3	40.1 ±9.2	42.1 ±9.5	53.5 ±10.9	50.9 ±12.9	61.1 ±12.8
10	8.4 ±4.0	20.3 ±5.8	25.6 ±6.6	34.2 ±8.6	40.4 ±11.8	50.7 ±17.2	50.9 ±19.8	60.5 ±19.0
Positive control – cyclophosphamide	-	19.8 ±7.4	-	33.8 ±7.7	-	53.2 ±14.9	-	50.4 ±16.5

[a] Data were obtained from pages 43 to 45 (Table 6) of the study report. Mean body weight gains of the test substance treatment and positive control groups were not statistically compared with those of the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Mean food consumption for the control and test substance treatment groups during the test substance treatment period were considered comparable to that of the control. The mean food consumption for the control and the test substance treatment groups is presented in Table 4.

TABLE 4 Average food consumption during the treatment period [a]

Treatment group (mg/kg/day chlorpyrifos)	Mean food consumption (grams/day)				
	Days 1-4	Days 4-8	Days 8-15	Days 15-22	Days 22-29
Females (n=10/group)					
0 (Control)	16.4±1.4	17.0±1.5	16.9±1.6	17.3±1.5	16.1±1.8
0.4	16.6±0.8	16.6±1.0	16.7±1.1	16.7±0.7	16.1±1.0
2	16.3±0.9	17.5±1.2	17.4±1.3	17.3±1.1	17.2±1.7
10	15.9±1.4	16.8±1.1	16.9±1.2	16.9±1.7	17.5±2.4

[a] Data were obtained from page 47 (Table 7) of the study report.

2. **Test substance consumption:** The average daily doses of chlorpyrifos calculated for the control and test substance treatment group are presented in Table 5.

TABLE 5 Test substance intake[a]

Treatment group (mg/kg/day chlorpyrifos)	Dose / adjusted dose [b] (mg/kg/day)					
	Days 1-4	Days 4-8	Days 8-15	Days 15-22	Days 22-29	Time-weighted average [c]
Females						
0 (Control)	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0
0.4	0.406 / 0.174	0.380 / 0.217	0.447 / 0.447	0.421 /0.421	0.405 / 0.405	0.416
2	2.003 / 0.859	2.006 / 1.15	2.304 / 2.30	2.118 / 2.12	2.081 / 2.08	2.13
10	9.78 / 4.19	9.78 / 5.59	12.1 / 12.1	10.5 / 10.5	10.4 / 10.4	10.7

[a] Data were obtained from page 48 (Table 8) of the study report.

[b] Dose = [ppm x food consumption (mg/day) for that period] divided by [average body weight (kg) for that period]
Adjusted dose = dose (mg/kg/day) x factor for that period. A factor is used to adjust for the time period in which a concentration is fed (may be equal to, greater than or less than 7 days, where a factor of 1 = 7 days)

[c] Sum of the adjusted mg/kg/day periods divided by the sum of all factors

3. **Food efficiency:** Food efficiency was not determined.

D. SACRIFICE AND PATHOLOGY:

1. **Anatomic pathology:**

Gross pathology: There were no gross lesions observed in the test substance-treatment groups that were considered treatment-related. All observations were considered spontaneous. All animals in the positive control group had decreases in thymus and spleen sizes. The gross pathological observations are summarized in Table 6.

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TABLE 6 Gross pathological observations[a]

Pathological Observation	Number of observances				
	Treatment group (females, n=10/group)				
	Control	0.4 mg/kg/day	2 mg/kg/day	10 mg/kg/day	Positive control
Skin and subcutis					
Scab; back/thorax; focal	0	0	0	1	0
Scab; neck; multifocal	0	1	0	0	0
Stomach					
Decreased ingesta	6	4	1	3	4
Thymus					
Decreased size	0	0	0	0	10
Hemorrhage	0	1	0	0	0
Spleen					
Decreased size	0	0	0	0	10

[a] Data were obtained from pages 55 to 58 (Table 14) of the study report.

Organ weights: There were no statistically significant differences between the mean terminal body weights or the mean absolute and relative (organ to terminal body weight ratio) spleen and thymus weights for the test substance treatment groups and the control. For the positive control, absolute and relative mean spleen and thymus weights were significantly lower than the control ($p \leq 0.05$); these results were expected. Mean absolute and relative spleen weights were decreased 51.4% and 49.3% of the control value, respectively. Mean absolute and relative thymus weights were decreased 77.1% and 76.2 % of the control value, respectively. Relative and absolute spleen weights and terminal body weights are presented for all groups in Table 7.

TABLE 7 Terminal body, spleen, and thymus weights[a]

Treatment group (mg/kg/day chlorpyrifos)	Mean weight				
	Terminal body weight (grams ±SD)	Spleen		Thymus	
		Absolute weight (grams ±SD)	Relative weight (%)	Absolute weight (grams ±SD)	Relative weight (%)
Females (n=10/group)					
0 (Control)	229.4±20.2	0.527±0.042	0.231±0.025	0.424±0.070	0.185±0.027
0.4	229.9±11.3	0.500±0.087	0.217±0.035.	0.400±0.082	0.174±0.036
2	232.7±15.0	0.516±0.132	0.221±0.047	0.429±0.125	0.183±0.045
10	233.5±26.0	0.548±0.069	0.236±0.028	0.430±0.099	0.183±0.031
Positive control – cyclophosphamide	219.3±19.5	0.256*±0.041	0.117*±0.017	0.097$±0.021	0.044$±0.007

[a] Data were obtained from pages 53 and 54 (Table 13) of the study report.
* Statistically different from the control ($p \leq 0.05$) using Dunnett's test.
$ Statistically different from the control ($p \leq 0.05$) using Wilcoxon's test.

Histopathology: Histopathological examinations were not performed.

2. Clinical pathology:

Hematology: There were no test substance treatment-related effects on any of the hematological parameters examined. The results of the differential WBC count are presented in Table 8.

TABLE 8 Differential white blood cell (WBC) summary [a]						
Treatment group (mg/kg/day chlorpyrifos)	Mean percent (±SD) [b]					
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Large unstained cells [c]
Females (n=10/group)						
0 (Control)	14.7±9.2	82.3±9.5	1.3±0.4	1.0±0.3	0.2±0.1	0.7±0.2
0.4	16.0±6.5	80.3±7.0	1.6±0.7	1.3±0.5	0.2±0.1	0.7±0.3
2	13.3±6.4	83.8±6.6	1.2±0.6	0.9±0.2	0.2±0.0	0.6±0.2
10	14.2±6.4	81.7±6.8	1.9±0.6	1.4±0.5	0.2±0.1	0.7±0.2

[a] Data were obtained from page 50 (Table 10) of the study report.
[b] The means for the treatment groups were not statistically compared with the means for the control.
[c] Includes atypical lymphocytes, large lymphocytes, plasma cells and blasts

Cholinesterase activity: Both RBC and brain ChE activity were affected by test substance treatment. RBC ChE activity was decreased to a greater extent and at lower dose levels than brain ChE activity. RBC ChE activity was significantly decreased ($p \leq 0.05$) at all dose levels; mean activities were 53.7%, 0.9% and 0.2% of the control value for the 0.4, 2, and 10 mg/kg/day treatment groups, respectively. Mean brain ChE activity was significantly decreased ($p \leq 0.05$) for the 2 mg/kg (91.3% of control) and 10 mg/kg/day (18.2% of the control) groups only. The inhibition observed in the present study was said to have been generally consistent with previous chlorpyrifos studies. The results of the ChE activity assays are presented in Table 9.

TABLE 9 Cholinesterase (ChE) activity [a]				
Treatment group (mg/kg/day chlorpyrifos)	Red Blood Cells		Brain	
	Mean ChE activity (U/L±SD)	Percent of control	Mean ChE activity (U/L±SD)	Percent of Control
Females (n=10/group)				
0 (Control)	5241±626.9	100.0	50057±1686.5	100.0
0.4	2814*±504.9	53.7	50393±2096.9	100.7
2	50*±53.8	0.9	45693*±2534.2	91.3
10	10*±0.0	0.2	9112*±1537.9	18.2

[a] Data were obtained from pages 51 and 52 (Table 11and Table 12) of the study report
* Statistically different from the control ($p \leq 0.05$) using Dunnett's test

E. IMMUNOTOXICOLOGY:

a. Anti-SRBC IgM response (ELISA)

Statistical analysis indicated no significant differences between the anti-SRBC IgM titers of the test substance treatment groups and the control. The mean anti-SRBC IgM titers of the 2 and 10 mg/kg/day treatment groups were 64% and 41% lower than the control value, respectively. However, there was no consistent dose-response for the 3 test substance treatment groups. The investigators believe that the decreased response for the mid- and high-dose groups may be partially due to an elevated mean for the vehicle control; 4 animals in the control group had high (>110,00 u/mL) antibody titers (maximum value of 183,609) that were outside of values observed for recent historical control data. Maximum values of 37,908; 30,976; and 105,850 u/mL were reported for controls in 3 historical studies (page 29, Text Table 3). Two animals in the 0.4 mg/kg/day group also had high antibody titers (maximum value of 153,236 u/mL). Individual anti-SRBC IgM data were presented on

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pages 140-142 (Appendix Table 11) of the study report. The distribution of individual animal data did not demonstrate any trend or suppression among treatment and control groups. The positive control demonstrated a significant (>99%) reduction in the anti-SRBC IgM response compared with the diet control. The results of the ELISA are presented in Table 8.

TABLE 8 Results of the ELISA[a]

Treatment group (mg/kg/day chlorpyrifos)	Mean anti-SRBC IgM titer (u/mL±SD / SE)
Females (n=10)	
0 (Control)	75647 ± 71973 / 22760
0.4	69436 ± 47916 / 15152
2	27302 ± 30762 / 9728
10	44953 ± 18011 / 5696
Positive control - cyclophosphamide	283[$] ± 209 / 66.04

SD/SE = standard deviation /standard error

[a] Data were obtained from pages 59 and 60 (Table 15) of the study report.

[$] Statistically different from the control ($p \leq 0.05$) using Wilcoxon's test.

b. NK cell activity assay: Not performed.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators conclude there were no treatment-related effects of chlorpyrifos treatment on body weight, body weight gain, food consumption, hematology, or absolute and relative spleen and thymus weights. RBC and brain cholinesterase activities were inhibited by treatment in a dose-dependent manner. RBC cholinesterase activity was significantly decreased at all dose levels when compared with the control; brain cholinesterase activity was unchanged at 0.4 mg/kg/day, but was significantly decreased at the 2 and 10 mg/kg/day dose levels

The investigators state that "a clear assessment of the antibody response could not be made." Although there were no statistically significant differences between the anti-SRBC IgM titers of the substance treatment group and the control, the titers for the 2 and 10 mg/kg/day treatment groups were decreased 64% and 41%, respectively, when compared with the control. The decreased response in these dose groups may have been due, in part, to a high mean value for the control group. The biological significance of these observations also was confounded by the lack of a clear dose response (the decrease was greater for the mid-dose group than for the high-dose group). In the present study there were no test substance treatment-related effects on spleen and thymus weights or on hematological parameters that would suggest the potential for immunotoxicity. Previous repeat-dose studies (2-week, 28-day, 90-day, 2-year) studies in rats and mice on file with EPA to meet registration guideline requirements also have not indicated the potential for immunotoxicity based on the absence of effects on the spleen, thymus, hematological parameters, and histopathology.[3] (*Note*: The investigators also identified a study in the

[3] References cited (not available to the reviewer): 1) Crown, S., A. Weiss, T. Nyska, et al. (1987) Pyrinex technical-toxicity in dietary administration to mice for 13 weeks. Life Science Research Israel Ltd., Laboratory Project ID MAK/105/PYR; 2) Davies, D., J. Tollett, and L. Lomax (1985) Chlorpyrifos: a four-week dietary study in CD-1

published literature that evaluated the anti-SRBC antibody response to a single dose level of 5 mg/kg chlorpyrifos; although there was a decrease in the antibody forming cell response when expressed per 10^6 splenocytes, there was no effect when expressed per spleen.[4] The authors of the study concluded that the overall effect of chlorpyrifos on antibody production was of minimal significance. Several deficiencies were noted for this study, among which were a non-standard dosing schedule, lack of multiple dose levels, and absence of a positive control.) The investigators conclude that the evidence for the immunotoxicity of chlorpyrifos was equivocal. This interpretation was said to be consistent with the interpretation categories for immune system toxicity suggested by the National Toxicology Program.[5]

B. REVIEWER COMMENTS:

Although the investigators consider the lower (not statistically significant) mean body weights and body weight gains seen in the positive control group to be due to normal body weight variability, the reviewer does not believe that a treatment (cyclophosphamide)-related effect can be ruled out. It may also be noted that although the investigators state that the slightly lower body weights and body weight gains observed in the 10 mg/kg/day treatment group had limited biological significance, they never state whether they consider the decreases potentially treatment-related.

It was stated in the report that blood was collected for the assessment of ChE activity in RBCs and plasma (page 29, Clinical Pathology). Although plasma samples were frozen and stored for "analysis if needed" (page 21, Assessment of RBC Cholinesterase Activity), there were no data for plasma ChE activity in the study report.

For systemic toxicity related to treatment with chlorpyrifos, the NOAEL for female rats is 10 mg/kg/day (highest dose tested), based on no effects were seen in clinical observations, body weight, food consumption, and hematological parameters. The LOAEL for systemic toxicity was not established. The LOAEL for neurotoxicity is 0.4 mg/kg/day based on decreased RBC cholinesterase activity. A NOAEL for neurotoxicity was not established (i.e., lower than the lowest dose tested).

For immunotoxiocity, the anti-SRBC IgM titers did not show statistically significant differences among treatment and the control groups. The titers for the 2 and 10 mg/kg/day

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mice. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan; 3) Liberacki, A., W. Breslin, D. Dittenber, et al. (1990) Chlorpyrifos: two week dietary probe study in Sprague-Dawley rats. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan; 4) Szabo, J., J. Young, and M. Grandjean (1988). Chlorpyrifos: 13 week dietary toxicity study in Fischer-344 rats. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan; 5) Warner, S., C. Gerbig, R. Strebing, et al. (1980) Results of a 2-year toxicity and oncogenic study of chlorpyrifos administered to CD-1 mice in the diet.; and 6) Young, J. and M. Grandjean (1988) Chlorpyrifos: 2-year dietary chronic toxicity-oncogenicity study in Fischer-344 rats. Szabo, J.R., J.T. Young, and M. Grandjean (1988). Chlorpyrifos: 13 Week dietary toxicity study in Fischer-344 rats. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

[4] Blakley, B., M. Yole, J. Brousseau, et al. (1999) Effects of chlorpyrifos on immune function in rats. Veterinary and Human Toxicology 41:140-144.

[5] NTP (National Toxicology Program) (2009), Explanation of Levels of Evidence for Immune System Toxicity. U.S. Department of Health and Human Services.http://ntp.niehs.nih.gov/index.cfm?objectid=070CBE5C-CDE4-8891-9534AE31C66CF2CD.

treatment groups were decreased (64% and 41%, respectively) when compared with the control. The study author considered the decreased response in these dose groups may have been due, in part, to a high mean value (75647 u/ml) for the control group. The mean historical control values were ranged 8614 to 22067 u/ml with maximum values of 37908, 30976, and 105850 u/mL (only three studies were provided). The biological significance of these observations also was confounded by the lack of a clear dose response (the decrease was greater for the mid-dose group than for the high-dose group). The distribution of individual animal data did not demonstrate any trend or suppression among treatment and control groups. There was no evidence demonstrated significant suppression of the anti-SRBC IgM response in animals treated with chlorpyrifos. The positive control data had demonstrated the validity of the assay.

The NK cell activity was not evaluated. Evaluation of repeat-dose studies (2-week, 28-day, 90-day, 2-year) studies in rats and mice showed no treatment-related effects on spleen and thymus weights and histopathology parameters that would suggest the potential for immunotoxicity. Under the HED guidance, if the TDAR assay is negative and evaluation of observational endpoints from all available toxicology database provide no evidence of immunotoxicity, the test article is considered negative for immunotoxicity and evaluation of NK activity is not necessary.

For immunotoxicity related to treatment with chlorpyrifos, the NOAEL for female rats is 10 mg/kg/day (highest dose tested) based on the overall weight-of-evidence. A lack of dose-related response for anti-SRBC IgM titers at the mid- and high-dose levels, a lack of statistical significance at any dose level, and a lack of evidence of other immunological effects (absolute and relative spleen and thymus weights, hematological parameters). A LOAEL for immunotoxicity was not established.

C. STUDY DEFICIENCIES:

There were no major study deficiencies in study design or methods. The study did not identify a definitive NOAEL/LOAEL for immunotoxicity. A repetition of the anti-SRBC IgM response assay might help to clarify the results.

Minor deficiencies were as follows:

- A physical description of the test substance was not provided.
- The stability of the test substance as supplied was not provided.

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June 30, 2010

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Attn: Yan Donovan (7505P)

AI: CHLORPYRIFOS
CHEMICAL CODE: 059101
DATA SUBMISSION IN SUPPORT OF REGISTRATION REVIEW

Enclosed are final reports for several studies being submitted in support of EPA's reevaluation of chlorpyrifos as part of the Registration Review program and in anticipation of an impending data call-in. Submission of final reports for the comparative cholinesterase assay (CCA) study and inhalation toxicity study follows the earlier submission of data and interim reports on March 30, 2010. Please note that, in addition to the core CCA study report, we are also submitting a BMD modeling assessment of the new CCA data and existing endpoint data to assist the Agency's evaluation efforts. Finally, a completed immunotoxicity study report is also being submitted. Dow AgroSciences notes that study protocols for the CCA, inhalation, and immunotoxicity studies were all earlier submitted to the Agency and received positive reviews from the Health Effects Division.

Contents of Submission

Volume	Contents
Volume 1 (Administrative)	▪ Transmittal document (this letter) ▪ General Summaries for Public Release (2)

Volume Guideline	MRID NO.	Contents/Study
Volume #2 (Part 1 of 2)(Part 2 of 2) N/A	**48139301**	Comparison of Cholinesterase (ChE) Inhibition in Young Adult and Preweanling CD Rats After Acute and Repeated Chlorpyrifos or Chlorpyrifos-Oxon Exposures Authors: M.S. Marty and A.K. Andrus Report Date: June 29, 2010 Study ID: 091107 Pages: 1-1062 (3 copies)

Volume Guideline	MRID NO.	Contents/Study
Volume #3 N/A	**48139302**	Benchmark Dose Modeling for Cholinesterase Inhibition of Chlorpyrifos and Chlorpyrifos-Oxon Authors: R. Reiss Report Date: June 30, 2010 Study ID: 0900956.000 B0T0 06010 Pages: 1-138 (3 copies)

Volume Guideline	MRID NO.	Contents/Study
Volume #4 N/A	**48139303**	Acute Inhalation Exposure of Adult CD Rats to Particulate Chlorpyrifos Aerosols: Kinetics of Concentration-Dependent Cholinesterase (ChE) Inhibition in Red Blood Cells, Plasma, Brain, and Lung Authors: J. A. Hotchkiss et al. Report Date: June 29, 2010 Study ID: 091113 Pages: 1-427 (3 copies)

Volume Guideline	MRID NO.	Contents/Study
Volume #5 870.7800	**48139304**	Chlorpyrifos: Assessment of Immunotoxic Potential Using the Sheep Red Blood Cell Assay After 28-Day Dietary Exposure to Female Crl:CD(SD) Rats Authors: D. R. Boverhof et al Report Date: June 28, 2010 Study ID: 101023 Pages: 1-174 (3 copies)

If you require additional information, please contact me or Joyce Carroll, Registration Assistant for this product, at phone (317) 337-4631.

Sincerely,

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Enclosures

KDR/jc