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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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Memorandum

SUBJECT: Toxicology Chapter for Chlorpyrifos. DP Barcode D255714, Case 818975, PC Code 059101.

FROM: Deborah Smegal, M.P.H. Toxicologist
Re-Registration Branch 3
Health Effects Division (7509C)

THRU: Jess Rowland, M.S., Branch Chief
Re-registration Branch 3
Health Effects Division (7509C)

TO: Mark Hartman, Chemical Review Manager
Special Review and Reregistration Division (7508C)

This memorandum summarizes the guideline studies submitted by the registrant, and other relevant toxicity studies considered by HED in developing the acute and chronic reference doses (RfDs) and toxicity endpoints for use in risk assessment.

Table 1. Acute Toxicity Results for Technical Chlorpyrifos			
STUDY	MRID Number	RESULTS	CATEGORY
Dermal Irritation - rabbit	Accession No. 112115	slight irritation (slight hyperemia and burns that healed by 21 days)	III
Dermal Sensitization - guinea pig	00095497	non-sensitizing	NA
Acute Delayed Neurotoxicity in hens	00097144 00405106	not neurotoxic at 50, 100 or 110 mg/kg	NA

NA = not applicable

b. Subchronic Toxicity

Several subchronic studies are available for chlorpyrifos including two oral rat studies, one oral dog study, a 21 day dermal toxicity study in rats, and two inhalation studies in rats. The most sensitive toxicological endpoint following subchronic oral exposure is inhibition of plasma and red blood cell cholinesterase in dogs at 0.22 mg/kg/day (Barker 1989) and plasma inhibition in rats at doses as low as 0.025 mg/kg/day (Crown et al. 1985). Rats exposed to higher doses also exhibited increased brain and heart weight, adrenal gland effects and decreased body weight gain at 1 mg/kg/day and hematological alterations suggestive of anemia at higher doses of 10 mg/kg/day (Szabo et al. 1988). No adverse effects were noted in rats exposed via inhalation to the highest attainable vapor concentration of 20.6 ppb (287 $\mu g/m^3$) (Corley et al. 1986a,b, Newton 1988). No adverse effects were observed in the 21-day dermal study in rats at doses as high as 5 mg/kg/day. However, in a 4-day dermal probe study, rats dermally exposed to doses of 0, 1, 10, 100, or 500 mg/kg/day exhibited reductions in plasma and red cell cholinesterase activities at doses of 10 to 500 mg/kg/day. The 21-day dermal NOAEL is 5 mg/kg/day based on a 45% and 16% inhibition of plasma and red blood cell cholinesterase, respectively in rats dermally exposed to 10 mg/kg/day for 4 days (MRID No. 40972801; Calhoun and Johnson, 1988). The following table summarizes the subchronic toxicity studies for chlorpyrifos:

Table 2. Subchronic Toxicity of Technical Chlorpyrifos			
GDLN	**STUDY**	**DOSE (mg/kg/day) (1)**	**RESULTS (mg/kg/day) (1)**
82-2	21-Day Dermal Toxicity Study in Rats and 4-day Dermal Probe Study MRID #: 40972801 Calhoun and Johnson 1988 Core Grade: acceptable guideline	0, 0.1, 0.5, 1 or 5 (21 day study) 0, 1, 10, 100 or 500 (4-day dermal probe study)	**100% pure chlorpyrifos** NOAEL: 5 (plasma and RBC ChEI) LOAEL: 10 (45% plasma and 16% RBC ChEI following 4 days of exposure) NOAEL (systemic): not identified LOAEL (systemic): not identified (>5) Effects: Slight erythema in 2/4 females at 1 and 10 mg/kg/day, respectively.
82-4	Subchronic Inhalation in Rats (90 days) (nose only) MRID #: 40013901 & 40166501 Corley et al. 1986a,b Core Grade: acceptable guideline	0, 5.2, 10.3 or 20.6 ppb (0, 72, 143 or 287 $\mu g/m^3$) (maximum dose equivalent to 0.044-0.082 mg/kg/day)	**100% pure chlorpyrifos** NOAEL: not identified (ChEI and systemic) LOAEL: not identified at highest attainable vapor concentration (>20.6 ppb or 287 $\mu g/m^3$) (ChEI and systemic)
82-4	Subchronic Inhalation in Rats (90 days) (nose only) MRID #: 40908401 Makhteshim-Agan; Newton 1988 Core Grade: acceptable guideline	0, 5, 10 or 20 ppb (0, 70, 143 or 287 $\mu g/m^3$) (equivalent to 0, 0.024, 0.048 or 0.097 mg/kg/day, respectively)	**95% a.i. chlorpyrifos** NOAEL: not identified (ChEI and systemic) LOAEL: not identified at highest attainable vapor concentration (>20 ppb) (ChEI and systemic)

(1) Unless specified.
ChEI = Cholinesterase Inhibition
RBC = red blood cell
NOAEL = No Observable Adverse Effect Level
LOAEL = Lowest Observable Adverse Effect Level

c. Chronic Toxicity and Carcinogenicity

Chlorpyrifos was evaluated for carcinogenic potential in both rats (2 studies), and mice (2 studies). There was no evidence of treatment-related tumors or carcinogenicity. In addition, chlorpyrifos was evaluated for chronic toxicity in dogs. In all animal species, the most sensitive toxicological endpoint is inhibition of plasma, red blood cell and brain cholinesterase that occurred at levels in the range of 0.03 to 1 mg/kg/day. Dogs appear to be the most sensitive species for cholinesterase inhibition and systemic effects, as noted by increased liver weights in dogs exposed to 3 mg/kg/day. Rats exposed to 7-10 mg/kg/day had decreased body weight and decreased body weight gain, ocular effects, adrenal gland effects and altered clinical chemistry and hematological parameters. Mice appear to be the least sensitive, as exposure to 45-48 mg/kg/day resulted in decreased body weight and an increased incidence of non-neoplastic

Table 3. Chronic Toxicity/Carcinogenicity of Technical Chlorpyrifos			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
83-2	*Chronic feeding study in* CD-1 mice (2 yrs) MRID # 00054352 & 00142902 (Accession No. 242059) Warner et al. 1980 Core Grade: acceptable guideline	0, 0.5, 5 or 15 ppm (highest dose tested is 2.25 mg/kg/day)	**99.6% a.i. *chlorpyrifos*** LOAEL: 2.25 (90%↓plasma, and 50%↓ RBC ChE activity relative to controls after 1 week) NOAEL(systemic) = 2.25 LOAEL (systemic): none observed (>2.25) Effects: no systemic effects observed at highest dose tested (HDT). No treatment-related tumors. ChE only measured at 15 ppm (2.25 mg/kg/day) after 1 and 4 weeks.
83-2	Chronic feeding/ carcinogenicity study in CD-1 mice (78 weeks) MRID # 42534201 Gur 1992 Core Grade: acceptable guideline	Males: 0, 0.89, 8.84, 45.2 Females: 0, 0.938, 9.79, or 48.1 (0, 5, 50 or 250 ppm)	**95.5% a.i. chlorpyrifos** NOAEL: none for ChEI LOAEL: 0.89 males; 0.938 females (significant 45-51% plasma ChEI in both sexes) NOAEL (systemic): 8.84 males, 9.79 females (50 ppm) LOAEL (systemic): 48.1 females, 45.2 males (HDT; 250 ppm) Effects: decreased body weight gain and food consumption in males, decreased water consumption in females, increased incidences of keratitis and hepatocyte fatty vacuolation, and increased incidence of gross clinical findings (ocular opacity and hair loss) in both sexes. Brain cholinesterase was inhibited at the high dose in both sexes. No evidence of carcinogenicity. Note: The validity of the RBC ChE assay is questionable.

ChEI = Cholinesterase inhibition

d. Developmental Toxicity

Chlorpyrifos was evaluated for developmental toxicity in rats, mice and rabbits. In one rat study, developmental effects (increased post-implantation loss) were noted at 15 mg/kg/day (HDT), that were also associated with maternal toxicity (Rubin et al. 1987a), while another rat study failed to observed developmental effects at 15 mg/kg/day (Ouellette et al. 1983). Developmental effects were also noted at higher doses in mice at 25 mg/kg/day (minor skeletal variations, delayed

Table 4. Developmental Toxicity of Technical Chlorpyrifos			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
83-3a	Developmental Study in CF-1 mice (gavage) MRID# 00095268 Deacon et al. 1979 Core Grade: Not acceptable guideline	0, 0.1, 1, 10, or 25 (gestation day 6-15)	**96.8% a.i. chlorpyrifos** Maternal NOAEL: 0.1 (plasma and RBC ChEI); 10 (systemic toxicity) Maternal LOAEL: 1 (plasma and RBC ChEI); 25 (systemic toxicity) based on decreased body weight, food and water consumption, and increased mortality. Developmental NOAEL: 1 (plasma and RBC ChEI); 10 for systemic toxicity Developmental LOAEL: 10 (plasma and RBC ChEI); 25 (systemic toxicity) based on minor skull variations, delayed ossification of skull bones and sternebrae and reduced fetal body length.
83-3 (b)	Developmental Study in New Zealand rabbits (gavage) MRID# 40436408 Makhteshim-Agan; Rubin et al. 1987b Core Grade: acceptable guideline	0, 1, 9, 81, or 140 (gestation day 7-19)	**96.1% a.i. chlorpyrifos** Maternal NOAEL: none observed for plasma ChEI; 81 for systemic toxicity Maternal LOAEL: 1 (plasma ChEI); 140 for systemic toxicity based on reduced food consumption, body weight loss, and apparent post-implantation loss. Developmental NOAEL (systemic): 81 Developmental LOAEL(systemic): 140 based on slightly decreased fetal weights and crown-rump lengths, and an increased incidence of unossified xiphisternum and/or 5th sternebra.
83-6	Developmental Neurotoxicity Study in Rats MRID: 44556901 Hoberman. 1998a,b Core Grade: not acceptable guideline, but upgradeable	0, 0.3, 1, or 5 (gestation day 6 through lactaction day 11)	**99.8% a.i. chlorpyrifos** Maternal NOAEL: none observed for plasma or RBC ChEI Maternal LOAEL: ≥0.3 (43%↓ plasma and 41%↓% RBC ChE activity relative to controls) Tentative Developmental NOAEL (systemic): 1 Tentative Developmental LOAEL(systemic): 5 (decreased body weight, and morphometric alterations in the brain of pups)

Table 5. Reproductive Toxicity of Technical chlorpyrifos			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
83-4	3-Generation Reproduction Toxicity in SD Rats MRID No: 00029064, 00064934 Thompson 1971 Core Grade: acceptable guideline	0, 0.03, 0.1,or 0.3 for first generation, and 0.1, 0.3 or 1 for second and third generation	Parental NOAEL: 0.1 Parental LOAEL: 0.3 (plasma and RBC ChEI) Reproductive NOAEL: >1 (HDT) Reproductive LOAEL: not identified

f. Mutagenicity Studies

Chlorpyrifos is not mutagenic in bacteria, or mammalian cells, but did cause slight genetic alterations in yeast and DNA damage to bacteria. Chlorpyrifos did not produce gene mutation in Ames reversion assays (MRID Nos.00157058 and 40436411) or in Chinese Hamster Ovary (CHO)/HGPRT assays in vitro (MRID Nos. 00152683 and 40436410). Also, it did not induce chromosome aberrations in vitro (MRID No. 40436409) and was not clastogenic in the mouse micronucleus test in vivo (MRID No. 00152684). Chlorpyrifos did not induce unscheduled DNA synthesis in isolated rat hepatocytes (MRID No. 00157057). A slight increase in recombination frequency in the Saccharomyces mitotic recombination assay (Accession No. 256040) and direct damage to DNA in a DNA repair assay using B. subtilis H17/m45 and E. coli pol A+/pol A- (Accession No. 256040) were noted. (These studies fulfill guidelines 84.)

g. Neurotoxicity

As noted previously, the principle and most sensitive toxic effect of chlorpyrifos is the inhibition of cholinesterase activity. A pharmacokinetic study in rats observed peak plasma cholinesterase inhibition of 28-40% 3-6 hours after exposure at 1 mg/kg (MRID No. 44648101), while plasma, red blood cell (RBC) and heart cholinesterase inhibition of 45%, 17% and 19%, respectively were observed in rats 24 hours following a single dose of 5 mg/kg (MRID No. 44273901). Clinical signs of neurotoxicity, in the absence of neuropathology, were observed in rats exposed to a single oral dose of 50 mg/kg as evidence by decreased motor activity, and increased incidence of clinical signs consistent with organophosphate intoxication. Chlorpyrifos was negative in the delayed neurotoxicity study in hens at single doses of 50, 100 (MRID 00097144) or 110 mg/kg (MRID 00405106). However, acute oral exposure to hens at 150 mg/kg/day caused >80% inhibition of neurotoxic esterase (NTE) 4 days after exposure (Capodicasa et al. 1991). In rats, chlorpyrifos failed to inhibit NTE at single doses up to 100 mg/kg. There is evidence that NTE inhibition is related to organophosphate-induced delayed neuropathy (OPIDN).

Table 6. Neurotoxicity of Technical Chlorpyrifos			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
82-8	13 Week Rat Neurotoxicity Study MRID 42929801 Shankar et al. 1993 Core Grade: acceptable guideline	0, 0.1, 1, 5, or 15	**98.2% a.i. chlorpyrifos** NOAEL (systemic): ≥15 LOAEL (systemic): none established Effects: Decreased motor activity and an increased incidence of urine incontinence in females. Note: This study did not measure cholinesterase activity.
NA	Special Acute Neurotoxic Esterase (NTE) Rat Study *MRID 44273901* Dittenber 1997 Core Grade: acceptable non-guideline	0, 1, 5, 10, 50 or 100	**98.1% a.i. chlorpyrifos** NOAEL: 1 [plasma ChE, and RBC and heart acetyl cholinesterase (AChE)] *LOAEL: 5 (45% plasma ChEI; 17% RBC* AChEI; and 19% heart AChEI). Effects: NTE was not inhibited at any dose. Note: cholinesterase measurements were made 24 hours post exposure.
NA	Cognitive Rat Study MRID 44020901 Maurissen et al. 1996 Core Grade: Acceptable non guideline	0, 1, 3, or 10 for 5 days/week for 4 weeks	**98.1% a.i. chlorpyrifos** NOAEL: none observed (plasma and RBC ChE), LOAEL: 1 (68% plasma ChEI; 56% RBC ChEI and 8% brain ChEI). NOAEL (systemic): 1 (miosis) LOAEL (systemic): 3 (miosis)
83-6	Developmental Neurotoxicity Study in Rats MRID: 44556901 Hoberman. 1998a,b Core Grade: not acceptable guideline, but upgradeable	0, 0.3, 1, or 5 (gestation day 6 through lactaction day 11)	**99.8% a.i. chlorpyrifos** Maternal NOAEL: none observed for plasma or RBC ChEI Maternal LOAEL: ≥0.3 (43%↓ plasma and 41%↓% RBC ChE activity relative to controls)

NA = Not applicable

Acute Neurotoxicity Studies

The acute neurotoxicity of chlorpyrifos was evaluated in rats. Rats were exposed once by gavage to 0, 10, 50 or 100 mg/kg/day chlorpyrifos and evaluated for neurotoxicity on days 1 (at peak

brain (>62%) cholinesterase activities. This study identifies a NOAEL ≥ 15 mg/kg, while a LOAEL was not established. (MRID 42929801). However, it should be noted that this study did not measure cholinesterase levels. This study satisfies guideline 82-7.

In a cognitive study (MRID 44020901) the effects of repeated oral administration of chlorpyrifos technical (purity, 98.1%) on the cognitive function of rats were evaluated with a delayed matching to position (DMTP) test. Groups of 10 *female* Long-Evans rats, pretrained in a DMTP apparatus were administered oral doses of chlorpyrifos in corn oil of 0, 1, 3, or 10 mg/kg/day for 5 days/week for 4 weeks. DMTP testing was conducted 6 days/week during treatment and continued post-dosing for 4 weeks. Testing for short-term memory (as evidenced by the retention rate) and attention/encoding deficits was based on the percent correct accuracy on several time delays. Slope over delay and intercept at time zero were calculated from these data for each rat and represented the "forgetting curve."

A satellite group of 6 rats/dose was sacrificed after the 4-week dosing period and plasma, erythrocyte and brain cholinesterase (ChE) were determined. Neurotoxic esterase (NTE) activity was determined in satellite rats from the control and high-dose groups one day after the last dose administration. Plasma (68%), RBC (56%) and brain (8%) ChE were inhibited at 1 mg/kg/day. At 3 mg/kg/day, plasma (83%), RBC (65%) and brain (63%) ChE inhibition was increased. At 10 mg/kg/day plasma (93%), RBC (65%) and brain (86%) ChE inhibition was further increased. NTE was minimally decreased (6%) in the high-dose group but this was not considered toxicologically significant. The LOAEL for ChE inhibition is < 1 mg/kg/day. No NOAEL was established.

The clinical sign of miosis was observed in rats that received 3 and 10 mg/kg/day particularly at weeks 3 and 4. Salivation and tremors were observed primarily at 10 mg/kg/day with the tremors usually disappearing by the following morning. The LOAEL for overt cholinergic signs is 3 mg/kg/day based on miosis. The NOAEL is 1 mg/kg/day.

A statistical analysis of the actual percent correct data was provided (supplemental report dated February 10, 1999) and no statistical differences (i.e., $p < 0.05$) indicative of treatment related decreases in percent correct choices were established for any dose or delay time. Thus, cognitive function is not obviously impaired. No consistent pattern in the intercept of the retention gradient was noted since it was increased at week 2 and decreased at week 3 but equivalent to the control at weeks 1 and 4 at 10 mg/kg/day. The DMTP parameters of actual total delay (increased by as much as 2.5 sec in the 0 delay trial at week 2), void trials per session (increased from about 5 in the control to about 15) and nosepokes (decreased ~42% at week 1 for the 15 sec delay) were affected in the 10 mg/kg/day chlorpyrifos dose at most or all intervals during dosing. Although these effects can be possibly related to a decrease in motor activity known to be associated with organophosphates, the increase in void trials may also indicate a motivational or attention deficit. The LOAEL for DMTP performance (i.e., increase in void trials) is 10 mg/kg/day. The NOAEL is 3 mg/kg/day.

PND 66-77.

Maternal toxicity in the high-dose (5 mg/kg/day) animals was manifested as increased signs of autonomic function toxicity, apparent at the end of gestation as fasciculations (6/25 treated vs 0/25 controls), and during early lactation (days 1-5) as fasciculations (16/24 treated vs 0/25 controls), hyperpnea (8/24 treated vs 0/25 controls), and hyperreactivity (17/24 treated vs 2/25 controls). Dams with all pups dying were increased in the high-dose group (3/23 treated vs 0/25 controls). There were no significant effects on bodyweight, food consumption, or pregnancy parameters. There were no unscheduled deaths in the maternal animals.

Brain ChE activity was decreased in the high-dose (↓90%) and mid-dose (↓18%) dams as compared to control. Erythrocyte (↓41-99%) and plasma (↓43-92%) ChE activities were decreased in a dose-dependent manner in all treated groups. **The maternal toxicity NOAEL was not observed. The maternal LOAEL was < 0.3 mg/kg/day, based on 43% and 41% plasma and RBC cholinesterase inhibition, respectively.**

For the F_1 generation pups, the high-dose group body weights were significantly reduced (↓8-15%) at PND 1 and 5 (pre- and post-culling). Body weights were also reduced from birth to PND 22 in Subset 4 high-dose animals (↓5-19%); body weight gains were reduced in these animals during the same period (↓5-30%). Additionally, compared to the controls, reduced terminal body weights were observed in the Subset 1 (PND 12) high-dose animals (↓17-19%) and the Subset 4 (PND 66) high-dose males (↓10%). For the F_1 generation adults, body weights of the high-dose males were decreased at PND 22 through 66 (↓11-17% vs controls). High-dose F_1 adult females also weighed less than controls at PND 22 (↓17% vs controls), but were of similar weight at PND 66. Body weight gains were also decreased in the high-dose males for the PND 22-40 interval (↓13% vs controls) and PND 40-66 interval (↓7%). Food consumption was decreased immediately after weaning (PND 23-30) in high-dose males and females (↓13% vs controls).

Development as assessed by pinna unfolding was delayed (4.0 days in treated vs 3.5 days in controls) in the high-dose group. Sexual maturation was delayed as assessed by time to preputial separation (106% of controls) and vaginal patency (103% of controls).

Pup viability was reduced as assessed by the following parameters: surviving pups/ litter (↓27%) and live litter size at culling (↓16%), pup viability index (↓29%), and pups found dead or presumed cannibalized (day 1 - 7.2 % treated vs 0.0% controls; days 2 to 5 - 24.7% treated vs 1.3% controls).

There were no statistically significant differences between the groups in the average acquisition and delay training. Additionally, there were no differences among dose groups when comparing retention of information during PNDs 23-25 and 62-92. Motor activity was decreased in high dose male and female pups on PND 14 (↓56% in males and ↓37% in females), and increased in high dose females on PNDs 18 and 22 (↑51% on both days). There was a statistically significant

additional 5 dams/dose and 5 pups/sex/dose on GD 20 and lactation days 1, 5, 11, 22, and 65 (pups only). ChE activity was determined in plasma, RBC, brain, and heart. For all analyses, samples were taken from dams and fetuses 4 hours postdosing, and from pups 2 hours postdosing of the dams.

No treatment-related clinical signs of toxicity in dams or pups and no differences in maternal body weights were observed at any time during the study. No differences in litter sizes at parturition or in the number of pups born dead were observed between the treated and control groups. Pup survival during lactation was similar for the treated groups as compared to the control.

Chlorpyrifos was detected in the blood of high-dose dams at a mean concentration of 108.78 ng/g on GD 20. Levels of chlorpyrifos then declined to 87% on lactation day 1, remained unchanged on lactation day 5, and were below the limit of detection by lactation day 11. Chlorpyrifos was detected at a low level (2.55 ng/g) in blood of mid-dose dams only on GD 20 and was not detected at any time in blood of low-dose dams. In milk, chlorpyrifos concentrations in the 0.3, 1.0, and 5.0 mg/kg/day groups were 20.57, 139.49, and 3022.00 ng/g, respectively on lactation day 1 and were 13.54, 81.76, and 1533.98 ng/g, respectively on lactation day 5. By lactation day 11, chlorpyrifos was detected only in the high-dose group at a level of 19.79 ng/g. Chlorpyrifos-oxon was not detected in the blood or milk of any dams at any time point.

Blood concentrations of chlorpyrifos in male and female fetuses from high-dose dams were 52.81 and 39.40 ng/g, respectively on GD 20. Concentrations in the pups declined to less than half of the GD 20 levels by lactation day 1 and were below the limit of detection by lactation day 5. Levels of chlorpyrifos in the blood of male and female fetuses from the mid-dose dams were 0.99 and 1.19 ng/g, respectively on GD 20, but were undetectable thereafter. Chlorpyrifos-oxon was detected in the blood of male and female fetuses from high-dose dams only on GD 20 at concentrations of 0.97 and 0.94 ng/g, respectively.

TCP was detected in the blood of dams from all treated groups on GD 20, lactation day 1, and lactation day 5. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood were 114.40, 322.01, and 1974.00 ng/g, respectively on GD 20, and were 142.93, 536.53, and 1449.92 ng/g, respectively on lactation day 5. On lactation day 11, TCP was detected only in the mid- and high-dose groups at levels of 9.87 and 71.40 ng/g, respectively.

TCP was detected in the blood of male and female fetuses from all dose groups in a dose dependent pattern on GD 20. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood on GD 20 were 93.93, 361.00, and 1680.00 ng/g, respectively for males, and were 99.49, 339.13, and 1884.00 ng/g, respectively for females. TCP was essentially not detectable in the blood of low- and mid-dose pups by lactation day 5. On lactation day 11, TCP was detected in the high-dose male and female pups at levels of 42.29 and 47.01 ng/g, respectively.

ChE activity in fore- and hindbrain from high-dose dams was 11.1-22.7% and 19.5-42.8%,

control group, 9 days for the 0.1 mg/kg/day group, 21 days for the 0.03 mg/kg/day and 28 days for the 0.014 mg/kg/day dose group. Blood was drawn twice pre-exposure for baseline values; twice weekly for plasma and red blood cell cholinesterase measurements, and once weekly for clinical chemistry and hematology. Urinalysis was also conducted weekly. The volunteers were also evaluated from 7 to 35 days post exposure.

After 1 and 3 days of treatment, the mean plasma cholinesterase activity was not significantly decreased at any dose level (up to 0.1 mg/kg/day) as compared to the baseline measurement. In the high dose group, plasma cholinesterase activity for individual subjects, relative to baseline, ranged from an 8% increase to a 41% decrease. However, significant 36-82% plasma cholinesterase inhibition relative to baseline was observed after 9 days of treatment with 0.1 mg/kg/day chlorpyrifos. In addition, one of the four men in the 0.1 mg/kg/day developed possible cholinergic clinical signs on day 8 (blurred vision, runny nose and a feeling of faintness). Exposure was discontinued on day 9 in this dose group due to plasma cholinesterase inhibition that exceeded the guideline of 20%-30%. No significant plasma ChE inhibition was observed in the men exposed to 0.03 mg/kg/day for 21 days (mean inhibition relative to baseline was 29%, with a range of 16-50%). A gradual recovery was observed in plasma ChE values equaling baseline values by day 25 of the recovery period. No effects on RBC ChE were found at any dose that could be attributed to treatment.

This study failed to control for confounding factors, such as smoking, and provided limited details on the study methodology and study design (i.e., no details on the volunteers, such as age or race, and there was no explanation why the 0.03 mg/kg/day dose group exposure was truncated at 21 days). It should be noted that the registrant contends that the clinical signs were attributed to a cold, and not chlorpyrifos exposure. HED believes that blurred vision is a typical cholinergic sign of cholinesterase inhibition, and can not be attributed to a common cold (Personal communication by Dr. Brian Dementi, Toxicologist, HED, OPP, January 29, 1996 with Dr. Jean Hollingsworth and Dr. Joe Bresee of the Center for Disease Control and Prevention, see February 2, 1998 HIARC Report, HED Doc No. 012471).

An acute oral and dermal pharmacokinetic study (Nolan et al. 1982, Accession No. 249203) dosed six men once with 0.5 mg/kg orally and four weeks later dosed five of these same men with 5 mg/kg dermally, and one man with 0.5 mg/kg dermally. Blood was collected 1, 2, 6, 12 and 24 hours, and up to 30 days (oral) and 9 days (dermal) post dosing for plasma and RBC ChE measurements. No signs or symptoms were observed in any of the subjects, but unlike the previous study, the primary focus of this study was pharmacokinetics. Men orally exposed to 0.5 mg/kg chlorpyrifos exhibited peak plasma ChE inhibition of 64-85%, 12 to 24 hours post-exposure and peak RBC ChE inhibition of 11-52% on post-exposure day 4. Men dermally exposed to 5 mg/kg chlorpyrifos exhibited peak plasma ChE inhibition of 27-45% on day 3, and mean RBC ChE inhibition of 8.6% on day 4. While RBC cholinesterase inhibition was judged not to be significantly affected in the orally dosed group, mean values for the group reached a low point on day 4 at 0.67 in comparison to pre-exposure control value of 0.92, a 27% decrease. Individual values varied on this day between 11-52% of their pre-dose controls and a paired t-test

plasma ChE activity was significantly inhibited approximately 28% and 40% relative to controls at 3 and 6 hours post exposure, respectively. By 12 hours post-exposure, plasma ChE activity was still significantly inhibited about 15%. The decrease in activity of rats treated with 50 or 100 mg/kg began within 10 minutes of treatment. By 12 hours after treatment, both groups were approximately 11% of the control group and had not shown signs of recovery.

Brain cholinesterase activity was not affected as dramatically by test material treatment as plasma activity with only the 10, 50, and 100 mg/kg dose groups showing significant effects. The brain cholinesterase activity of rats treated with 10 mg/kg test material began to decline within three hours of treatment and was significantly decreased by six hours after treatment. The brain cholinesterase activity in the 50 or 100 mg/kg dose groups decreased significantly within one hour of treatments; and by 12 hours, it was approximately 30% and 20%, respectively, of control. In none of the affected groups did brain cholinesterase show signs of recovery.

Peak chlorpyrifos blood concentrations occurred within three hours of treatment in all but the lowest dose group. The area under the curve (AUC) was calculated as 0.4, 1.1, 5.0, and 12.5 μmole hr L^{-1} for the 5.0, 10.0, 50.0, and 100 mg/kg groups, respectively and yielded calculated blood half-lives of chlorpyrifos of 2.7,1.5, 2.1, and 7.3 hours for the 5.0, 10.0, 50.0, and 100.0 mg/kg dose groups, respectively. Regardless of dose, the highest concentration of OXON detected was 2.5 ng/g found in the blood of rats treated with 50 mg/kg test material one hour post-treatment. Following treatment with 5 or 100 mg/kg labeled test material, ≥98% of the activity detected in the blood was identified as TCP metabolite with the remaining attributed to the parent compound. Since OXON is an intermediate in the formation of TCP and none of the metabolite was detected, these studies support that the half-life of the OXON metabolite is short (reportedly 10 seconds) and that *in vivo* metabolism of chlorpyrifos is rapid.

This study is considered acceptable (nonguideline). It may partially fulfill guideline requirements in other areas.

In addition, the cholinesterase and metabolite determination study in rats (MRID 44648102), which is the companion study to the developmental neurotoxicity study and was discussed previously in Section h, also evaluates the pharmacokinetics of chlorpyrifos in pregnant dams and pups.

k. Oral/Dermal Absorption

As discussed previously, the oral and dermal absorption of chlorpyrifos were evaluated in a human pharmacokinetic study (Nolan et al. 1982, Accession No. 249203). In this study, chlorpyrifos was administered by a single oral dose of 0.5 mg/kg to 6 human male subjects and dermally at 0.5 or 5.0 mg/kg to 5 of these 6 subjects. Based on the urinary excretion of the 3,5,6-TCP metabolite, the minimum absorption orally was approximately 70% and dermally, it was approximately 1-3%. After oral administration, the maximum plasma concentration of metabolite was 0.979 µg/ml 6 hours post-dosing, and dermally, 0.052 µg/ml 24 hours post-

administration of the test material, cholinesterase activity was assessed periodically over a 12-week period. The following is a quotation from the study report (P.253): "Cholinesterase inhibition in plasma was not as extensive as in either cortex or striatum at any time point during the observation period, but roughly equivalent rates of recovery of enzyme activity were noted between plasma and the brain regions." Inhibition in the striatum and cortex were essentially identical. Inhibition for these brain regions were 94-96%, 82-83%, 58-60% and approximately 20% at weeks 2, 4, 6 and 12, respectively. By comparison, plasma cholinesterase was inhibited at the same respective time points by about 90%, 55%, 30% and 0%. The authors advise that cholinesterase activities were not significantly different between treatment groups at the 12-week time point.

Although erythrocyte cholinesterase was not assayed in either of the above referenced publications, the data indicate that plasma cholinesterase inhibition correlated well with brain cholinesterase inhibition, and toxicity under certain conditions of each study. While in view of the author's discussion, this correlation may not hold to be true under all exposure scenarios, the correlation should be considered as substantive.

3. Padilla *et al.* (1994) correlations between plasma, whole blood and erythrocyte cholinesterase inhibition and brain cholinesterase inhibition were determined over a 35 day period following a single subcutaneous dose of Chlorpyrifos to Long Evans rats. The study revealed high correlation coefficients between inhibition of all three blood components enzymes and that of the frontal cortex during days 4-21 post-dosing. At the 35 day time point, plasma cholinesterase activity was less well correlated than was whole blood or erythrocyte cholinesterase activity with brain cholinesterase inhibition.

These three studies, collectively, reveal a good correlation between plasma and brain cholinesterase inhibition. In rats, at least in the Padilla study, erythrocyte cholinesterase appears to be more remarkably inhibited by Chlorpyrifos than either the plasma or brain enzyme activity. It is clear from the above discussion of the three animal studies that plasma cholinesterase inhibition has predictive value for brain cholinesterase inhibition in the case of Chlorpyrifos.

In the Coulston *et al.*(1972) study, plasma enzyme activity was reportedly inhibited at a lower dose than the erythrocyte cholinesterase activity. We have no explanation for this reversal of effect with respect to plasma and erythrocyte cholinesterase responses except that it may have to do with inherent differences between human and rat, or the circumstance of exposure. The limited number of subjects and variability of the cholinesterase assay methodology were also cited by the Committee as possible factors.

concluded that acute Chlorpyrifos exposure (subcutaneously injected) to dams during gestation produces more extensive neurological effects in the dam relative to the developing fetus.

2. Chanda and Pope (1996) examined the relative neurotoxicity of repeated, lower-level exposures to Chlorpyrifos during gestation in Sprague-Dawley rats. Doses of 6.25, 12.5, or 25 mg/kg/day of Chlorpyrifos were injected subcutaneously on gestation days 12-19. The dams and offspring were killed on gestation day 16 or 20 or postnatal day 3. No clinical signs of maternal toxicity were observed at any dose level; maternal body weight gain values were similar to control for all treated groups. Fetal body weight was similar to control for all treated groups, but a significant decrease in fetal body weight was observed on postnatal day 1 in the 25 mg/kg/day dose group. A significant dose-related inhibition of acetylcholinesterase was observed following the three dosing regimens at gestation day 20. In each case, maternal brain AChE inhibition was greater than the fetal brain AChE inhibition with all three doses. AChE inhibition (83-90%) was noted in maternal brain at all three collection times following repeated exposures at 25 mg/kg/day. Higher AChE inhibition (58%) was noted in fetal brain at gestation day 20 compared to 19-25% on postnatal day 3 in treated pups cross-fostered to control dams and in control pups cross-fostered to treated dams following repeated exposures at 25 mg/kg/day. Although similar reductions in brain muscarinic receptor binding were observed at gestation day 20 and postnatal day 3 in dams and developing brain between acute and repeated dosing regimens, greater changes in [^{3}H]cis-methyl dioxolane and [^{3}H]cytisine binding were observed following repeated exposures. Righting reflex and cliff avoidance tests were markedly altered following repeated exposures. The study authors concluded that the lower-level repeated exposures to Chlorpyrifos caused extensive neurochemical and neurobehavioral changes in developing rats in the absence of maternal toxicity (signs of clinical toxicity and body weight gain data). An additional conclusion that can be drawn from this study is that repeated dosing of Chlorpyrifos during gestation resulted in AChE inhibition in both dams and fetuses at dose levels as low as 6.25 mg/kg/day, and that the maternal response, as measured by brain cholinesterase inhibition on gestation day 20, was more severe than the fetal response.

B. Studies that address the comparison of the neurotoxic response of adults and neonatal or weanling animals include the following:

1. Pope et al. (1991) compared the time course of cholinesterase inhibition and recovery in whole brain between neonatal (postnatal day 7) and adult

not affected in either young or adult rats as a function of Chlorpyrifos exposure. After challenge with scopolamine (1 mg/kg by intraperitoneal injection) higher learning activity levels were observed in adult rats at 2, 4, 6, and 8 weeks after treatment; there was no similar increase in activity levels in treated neonatal rats. According to the study authors, these data suggested that although neonatal rats are more sensitive to acute lethal effects from high doses of Chlorpyrifos, adult rats exhibit more persistent neurochemical and neurobehavioral alterations following repeated, lower-level exposures.

4. In a study by Stanton et al. (1994), a single subcutaneous injection of Chlorpyrifos was administered to Long-Evans rat weanlings (21 days of age) at dose levels of 90, 120, or 240 mg/kg; T-maze delayed alternation was tested on postnatal days 23 or 26. Acetylcholinesterase activity and muscarinic receptor density (QNB binding) were determined in hippocampus and cortex of brains taken from pups 15 hours after the end of behavioral testing (the morning of postnatal days 24 and 27) Pups at 240 mg/kg showed signs of overt toxicity that precluded behavioral testing. Exposure to 120 mg/kg produced a transient selective memory impairment (a deficit in delayed alternation but not position discrimination) relative to the 90 mg/kg and vehicle groups. Exposure to Chlorpyrifos on postnatal day 21 produced dose-related inhibition and recovery of brain AChE over the postnatal day 24-27 age range. A similar pattern was observed in hippocampus. Binding of [^{3}H]QNB was reduced in frontal cortex on postnatal day 27 only at the 240 mg/kg dose. No significant effects were observed in the hippocampus. These results suggested to the study authors that the neurochemical effects of acute Chlorpyrifos administration are more transient and the behavioral effects are smaller and shorter-lived than what has previously been reported in adult rats.

C. One study further examined specific aspects of neurological toxicity in rats that were exposed postnatally:

1. Whitney et al. (1995) administered Chlorpyrifos by subcutaneous injection to neonatal rats in apparently subtoxic doses that cause no mortality and little or no weight deficits. Developing brain regions (cerebellum, forebrain, and brainstem) were examined. One-day old rats showed significant inhibition of DNA and protein synthesis in all brain regions within 4 hours of treatment with 2 mg/kg. In comparison, when 0.6 µg Chlorpyrifos was administered directly to the brain via intracisternal injection, equivalent results were observed; this indicates that the inhibition in DNA synthesis was not secondary to systemic toxicity and

maternal blood and brain and fetal brain ChE activity was assayed. Remaining litters were allowed to deliver. Animals were killed on postnatal day 1, 4, 7, 12, 17, 21, and 92, and brains were dissected into 7 distinct regions for analysis of ChE activity, DNA and protein content, and serum thyroid hormone levels. Other developmental landmarks examined were eye opening (PND14-17), vaginal opening (PND32-45) preputial separation (PND 40-50, estrus cyclicity (PND 50-85), and testis weights (PND 92). Maternal weight gain, litter size, sex ratio, postnatal survival, and pup brain and body weights were not affected by late gestation Chlorpyrifos exposure. ChE activity was inhibited in both the maternal blood (60-80%) and brain (4-75%) on gestation day 19, whereas fetal brain ChE inhibition was $\leq$10%. There was no effect on the ontogeny of circulating thyroid hormones (serum T3 and T4), regional brain DNA or protein levels. Trends emerged in a dose-related fashion for eye opening, vaginal opening, and preputial separation. The study authors concluded that, in general, following late gestational exposure to Chlorpyrifos, the dam appears to protect the fetus from cholinesterase inhibition and from long-term adverse consequences.

3. Phillips *et al.* studied behavioral effects following exposure of Long-Evans rats to Chlorpyrifos (0, 3, or 5 mg/kg/day by gavage) on gestation days 14-18. Maternal effects were evaluated in the dams; there was a trend toward lower open field activity at 5 mg/kg/day. Offspring were evaluated for righting reflex on postnatal day 2-7, and 10 pups/dose/sex were tested for a range of neurobehavioral endpoints using a functional observation battery and motor activity assessments on postnatal days 17, 24, 65, and 92. On postnatal day 2, a trend towards slower righting reflex was evident in offspring of high-dose dams, but by postnatal day 7, all rats were righting normally. Few significant behavioral changes were detected at later time points. Male rats at 5 mg/kg/day showed decreased handling reactivity on postnatal day 24, and decreased activity and rearing in the open field testing throughout the course of testing. Female rats showed increased reactivity before weaning in the high-dose group and increased open field activity thereafter in the low-dose group. These data suggested to the study authors that there were qualitative sex-related differences associated with Chlorpyrifos exposure, but the effects were small. It was concluded that there were few persistent neurobehavioral consequences of Chlorpyrifos following late gestational exposure.

4. The effects of gestational exposure to Chlorpyrifos on the developmental profiles of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity in the rat brain was studied by Lassiter et al. It has been suggested that AChE has a role in the coordinated spatiotemporal development of the nervous system, and it was hypothesized that BuChE could also have a developmental function. Profiles of AChE and BuChE

2. Chanda *et al.* studied the developmental profiles of two organophosphate detoxifying enzymes, carboxylesterase (CaE; which can bind to OPs and reduce the effective concentration at the target enzyme site) and A-esterase (which can hydrolyze OPs to form nontoxic metabolites). Liver and plasma CaE and A-esterase activities were measured in Long Evans rats on postnatal days 1, 4, 76, 12, 17, 21, and 90. At postnatal day 1, liver and plasma CaE activities were 8 times lower and A-esterase activities were 11 and 35 times lower than that of adults. In general, as the rats developed, A-esterase appeared to mature faster than CaE. Enzyme levels were compared against the sensitivity of young rats to acute Chlorpyrifos exposure at various ages; during development, an inverse relationship between the enzyme activities and sensitivity to Chlorpyrifos toxicity was observed. It was concluded that a lack of these detoxifying enzymes in young rats could at least partially explain their increased sensitivity to Chlorpyrifos.

3. Mortensen *et al.* tested the hypothesis that young rats have less Chlorpyrifos-oxonase (CPFOase) activity than adults. CPFOase activity was measured in the brain, plasma, and liver of male postnatal day 4 and adult Long Evans rats. No brain CPFOase activity was measured at either age. Plasma and liver CPFOase activities were markedly lower (1/11 and ½, respectively) at postnatal day 4 compared to adult. To determine if the CPFOase activity could hydrolyze physiologically relevant concentrations of CPFO, the shifts in tissue AChE IC_{50} for CPFO in the presence or absence of CPFOase activity were compared. An increase in the "apparent" IC_{50} would be expected if CFPOase hydrolyzed substantial amounts of CPFO during the preincubation with CPFO. In the adult, both plasma and liver AChE "apparent" IC_{50} values were higher in the presence of CPFOase activity, suggesting that the CPFOase in those tissues was capable of hydrolyzing physiologically relevant concentrations of CPFO within 30 minutes. In young animals, however, there was less of a shift in the IC_{50} curves compared to the adult, confirming that the young animal has less capacity than the adult to detoxify physiologically relevant concentrations of CPFO via CPFOase.

4. In a further study by Mortensen, Hooper, and Padilla, the developmental profiles, kinetic parameters, and intrinsic (i.e., *in vitro*) sensitivity of male rat brain acetylcholinesterase were compared. The brains of postnatal day 4, 11, 17, 27, 40, or adult (PND 90) Long-Evans rats were collected, homogenized, and diluted to obtain approximately the same AChE activity for each age. Brain homogenates were incubated with varying concentrations of inhibitor (Chlorpyrifos-oxon, aldicarb, carbaryl, or maloaoxon), and AChE activity was measured. It was found that young and adult brain differed primarily in their specific activity; their K_ms, substrate profiles, and *in vitro* sensitivity to the selected anticholinesterase

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