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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **February 2, 1998**

MEMORANDUM

SUBJECT: **CHLORPYRIFOS - *FQPA REQUIREMENT* -** Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland, Executive Secretary Jess Rowland 2/2/98
Hazard Identification Assessment Committee
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman, K. Clark Swentzel 2/2/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Barbara Madden, Branch Senior Scientist
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

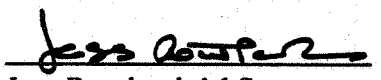
PC Code: 059101

The Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) met on December 11, 1997 to address the potential sensitivity of infants and children to exposure to Chlorpyrifos as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC also re-assessed the toxicology endpoints selected for dietary and non-dietary exposure risk assessments and determined the Uncertainty Factors (F's) and the Margins of Exposure (MOE's) for the various exposure scenarios. The HIARC met on January 22, 1998 to evaluate a report in which the Registrant proposed Reference Doses for acute and chronic exposures to Chlorpyrifos. The Committee's decisions are attached.

Committee Members in Attendance

Members present were William Burnam, Susan Makris, Nancy McCarroll, Melba Morrow, Kathy Raffaele, John Redden, Jess Rowland (Executive Secretary) and Clark Swentzel (Chairman). Member in absentia: Karl Baetcke.

Other HED staff present at the meeting were Alan Levy, Registration Action Branch 1, William Sette, Science Analysis Branch, Mary Clock, Risk Characerization and Analaysis Branch.

Report Preparation: 

Jess Rowland, M.S
Executive Secretary

1. BACKGROUND

The Reference Dose (RfD) for Chlorpyrifos, established by the Health Effects Division's RfD/Peer Review Committee on **February 21, 1986**, was verified by the Agency RfD Work Group on **March 11, 1986**, and was reaffirmed by the RfD Committee on **March 4, 1988**. On **September 8, 1993**, the RfD Committee met again and evaluated the toxicology data base and re-assessed the RfD for reregistration purposes. The Committee concluded that the RfD, established at 0.003 mg/kg/day should remain unchanged. The RfD was derived from a NOEL of 0.03 mg/kg/day based on plasma cholinesterase inhibition (ChEI) observed in human volunteers at the next higher dose level of 0.1 mg/kg/day (LOEL) and an Uncertainty Factor (UF) of 10 to account for the intra-species variability.

In a letter dated **April 25, 1995**, the Registrant petitioned the Agency to revise the RfD and, disputed 1) the validity of using plasma ChEI as a toxicological end-point for setting the RfD and 2) the NOEL established by the Agency for the human study (Chin and Nolan, 1995).

As for using plasma ChEI, the Registrant indicated that Chlorpyrifos causes plasma ChEI at much lower doses than that which is required to inhibit acetylcholinesterase found in red blood cells (RBC) and in brain. Therefore, the Registrant proposed that the Agency should reestablish the RfD based on RBC ChEI rather than plasma ChEI. The rationale provide by the Registrant for this approach are as follows:

1. Chlorpyrifos, like other organic phosphorothioates, exerts its effect by inhibition of acetylcholinesterase in the neuroeffector junction (e.g. synapse and myoneural junctions). Human plasma as pseudo-cholinesterase is entirely made up of butyrylcholinesterase, which is a different enzyme than acetylcholinesterase. Plasma butyrylcholinesterase has no known biological function and it plays no role in cholinergic transmission.

2. Human and animal studies have shown that Chlorpyrifos inhibits plasma cholinesterase at much lower doses than that which is required to inhibit acetylcholinesterase found in red blood cells and in brain.

3. Plasma cholinesterase activity is a poor predictor of cholinesterase activity in the target tissues of brain and muscle.

4. Although acetylcholinesterase found in erythrocytes does not play a role in cholinergic transmission, it is a better reflection of the acetylcholinesterase activity in brain, since the two enzymes are considered biochemically identical. In recent studies, cholinesterase activity in the RBC correlated better with the activity in brain and muscle than did cholinesterase activity in plasma.

5. Brain acetylcholinesterase is the best measure of the cholinergic effects of Chlorpyrifos. However, in the absence of data on brain acetylcholinesterase, human RBC values should be used to assess the toxicity of this chemical.

As for establishing the NOEL for the critical study, the Registrant postulated that the data in human volunteers indicate that 0.05 mg/kg/day should be the NOEL for deriving a single exposure RfD and 0.01 mg/kg/day should be the NOEL for repeated dietary and non-dietary (occupational) exposures to Chlorpyrifos. The following calculations were provided by the Registrant for establishing the Reference Dose(s):

1. Single exposure RfD: = 0.05 mg/kg/day derived from a NOEL of 0.50 mg/kg/day established for RBC cholinesterase inhibition in a human volunteer study and an UF of 10 to account for intra-species variability.

2. Repeated exposure RfD: = 0.01 mg/kg/day derived from a NOEL of 0.1 mg/kg/day for RBC ChEI in a human volunteer study and an UF of 10 to account for intra-species variability. Another approach for deriving the same RfD was suggested by using a NOEL of 1.0 mg/kg/day established in a chronic toxicity study in rats for brain cholinesterase inhibition and an UF of 100 for inter- and intra-species variations.

The RfD Committee met on **May 25** and on **November 23, 1995** to address the issues raised by the Registrant. In addressing the Registrant's rebuttal, the Committee was aware that the issue at hand was a multicomponent issue involving scientific, regulatory and policy aspects. The Committee indicated that its evaluation of cholinesterase inhibitors is usually performed on a case-by-case basis since anticholinesterase agents differ in their pharmacokinetics and pharmacodynamic aspects, and that the use of plasma ChEI in human studies in setting the RfD for Chlorpyrifos is supported by the weight of evidence as discussed below:

1. The Committee disagreed with the Registrant's assertion regarding the difference between acetylcholinesterase found in the erythrocyte and in the neuroeffector junctions (synapse and myoneural junctions) and plasma pseudo-cholinesterase (butyrylcholinesterase). It was the Committee's position that although the two esterases may vary in their substrate specificity, they are similarly susceptible to inhibition by organophosphorus pesticides and other cholinesterase inhibitors and that the two enzymes bind to inhibitors in the same manner.

2. The Committee agreed with the Registrant position in that both plasma pseudo-cholinesterase and erythrocyte cholinesterase have no known biological function and play no role in cholinergic transmission. However, the Committee disagreed with the Registrant statement that plasma cholinesterase activity is a poor predictor of cholinesterase activity in the target tissues of brain and muscle and that acetylcholinesterase found in erythrocytes is a better reflection of the acetylcholinesterase activity in brain.

4. Contrary to the Registrant's claim in the rebuttal letter, open literature information reviewed by the Committee demonstrated that, in the rat, plasma cholinesterase inhibition has been observed to correlate very well with brain cholinesterase inhibition following the administration of single doses of certain organophosphorus pesticides. In some cases organophosphorus pesticides caused brain cholinesterase inhibition at dose levels comparable to those causing inhibition of plasma cholinesterase without causing similar depression of the red blood cell cholinesterase, i. e., with certain organophosphorus pesticides there was a correlation between brain and plasma cholinesterase activities but not a good correlation between either brain and erythrocytes cholinesterase activities or plasma and erythrocytes cholinesterase activities (Pope and Chakraborti, 1992; Pope *et al.*, 1992; Padilla *et al.*1994). These studies are discussed in *Appendix A.*

5. Considering all weight of the evidence, the magnitude of plasma cholinesterase inhibition observed in the human study used by the Agency in setting the RfD for Chlorpyrifos was sufficient to define the NOEL and LOEL. In this study there were clinical signs, some of which are cholinergic in nature, accompanying plasma cholinesterase inhibition. Although, there was no evidence to suggest otherwise, other contributing factors such as common cold were not precluded and could have resulted in some, but not all, of these symptoms in some human subjects used in this study. For example, blurred vision observed in, at least, one subject is a typical cholinergic sign of cholinesterase inhibition and can not be attributed to 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 indicated that blurred vision is not a sign of cold /influenza. The Committee also indicated that although, no pronounced effect was noted on red blood cell cholinesterase in the human study, given the limited number of subjects used in this study and the variability of the cholinesterase assay, this kind of results are expected.

6. With respect to the Registrant proposal to use the rat study as an alternative for RfD setting, the Committee indicated that it is the Agency's position that the use of human data, when available, is preferred over animal data since it eliminates an unnecessary level of uncertainties, i.e. the need for inter-species extrapolation.

7. Finally, there were no new data submitted by the Registrant to the agency to change its position.

The RfD Committee, after thorough consideration of all aspects and based on all weight of the evidence, concluded that the RfD for Chlorpyrifos should remain unchanged. The factors described above were significant in shaping the Committee's position and conclusion on these issues. **(RfD Report dated 02/29/96).**

On **April 28, 1996**, the Health Effects Division's Toxicology Endpoint Selection Committee selected the doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments. but did not address the Margins of Exposure (MOEs) required for the various exposure scenarios (TES Document, dated 04/29/96).

On **May 9, 1997**, the Registrant submitted a comprehensive and succinct review of a wide variety of toxicology studies on Chlorpyrifos, title "*Proposed Reference Dose (RfD) for Acute and Chronic Exposure to Chlorpyrifos Based on the Criteria Described by the Acute Cholinesterase Risk Assessment Task Force and the Available Animal and Human Data*". This report was reviewed and endorsed by an external peer review committee convened by the Registrant. In this report, the Registrant proposed Reference Doses for Acute and Chronic exposures to Chlorpyrifos based on its analysis of the appropriate NOELs as well as Uncertainty Factors based on its own analyses of the studies and the criteria described by the Acute Cholinesterase Risk Assessment Task Force (ACRA) (MRID No. 44271001).

On **December 11, 1997** the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) 1) re-assessed the doses and endpoints selected for acute and chronic dietary as well as occupational/residential exposures, 2) determined the UF's and MOE's for the various risk assessments, and 3) addressed the sensitivity of infants and children to exposure to Chlorpyrifos as required by FQPA. On January 22, 1998, HIARC evaluated the above mentioned report (MRID No. 44271001) submitted by the Registrant.

This report supersedes the previous RfD and TES documents. Provided in this report is a comprehensive presentation of the dose and toxicological endpoints selected as well as the UF /MOE's employed for acute and chronic dietary as well as occupational/residential human risk assessments. Also provided is the Agency's response to the reference doses proposed by the Registrant for acute and chronic exposures to Chlorpyrifos.

II. HAZARD IDENTIFICATION

A. Acute Dietary (One-Day)

Study Selected: 28-Day Oral Toxicity - Human

MRID. Nos. 000112118 (Coulston *et al.*, 1972)

Executive Summaries: In a study conducted at Albany Medical College 5 healthy adult human males were given oral (tablet) doses of Chlorpyrifos at 0.014, 0.03 or 0.10 mg/kg. Blood was drawn twice pre-exposure; at 1, 3, 6 and 9 days after treatment for the 0.1 mg/kg/day dose group; and at additional posttreatment days for the 0.03 and 0.014 mg/kg/day dose groups. Plasma cholinesterase activity was measured. **No effects were seen on plasma cholinesterase activity after 1 and 3 days of treatment.** Plasma cholinesterase value was not significantly decreased at any dose level as compared to the baseline measurement. Significant inhibition of plasma cholinesterase activity was seen at this dose (0.1 mg/kg/day) only after 6 and 9 days of treatment. Therefore, for acute effects (i.e., single exposure), the NOEL was determined to be 0.10 mg/kg/day.

Dose and Endpoint for Risk Assessment: NOEL = 0.10 mg/kg/day based on lack of inhibition of plasma cholinesterase activity after 1 and 3 days of treatment.

Comments about Study and Endpoint: This dose was considered to be the NOEL and thus appropriate for this exposure period of concern (i.e., after a single dose) since cholinesterase inhibition was seen at this dose only after 6 and 9 days of dosing.

This risk assessment is required.

Acute Dietary Risk Assessment: The HIARC determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be retained** based on the following weight-of-the evidence considerations:

1. Chlorpyrifos is a neurotoxicant with evidence of OPIDN in humans and animals; there have been cases reported of neurophysiological effects in humans (See *Appendices A - E)*

2. Although no increased sensitivity was observed in young rats or rabbits following *in utero* exposure or in pups as compared to adults in the two-generation reproduction study in rats in the studies submitted under Subdivision F Guidelines increased susceptibility of offspring to the effects of Chlorpyrifos exposure has been identified in studies conducted in various reputable scientific research laboratories and reported in the open literature (See *Appendices A-E).*

3. There are data gaps for the assessment of functional development of young animals following pre- and/or postnatal Chlorpyrifos exposure and thus the need for a developmental neurotoxicity study.

4. There are a variety of uses for Chlorpyrifos that may involve exposure to infants and children.

Therefore, the HIARC determined that for acute dietary risk assessment, **a MOE of 100 is required** and includes a 10 x for intra-species variation and a 10 x for FQPA.

B. Chronic Dietary Risk Assessment (Reference Dose)

The RfD established in 1988 was re-assessed by the HIARC pursuant to the FQPA and is discussed below:

Study Selected: 28-Day Oral Toxicity - Human

MRID No. 00112118

Executive Summary: In a study conducted at Albany Medical College 5 healthy adult human males were given oral (tablet) doses of Chlorpyrifos at 0.014, 0.03 or 0.10 mg/kg. Blood was drawn twice pre-exposure; at 1, 3, 6 and 9 days after treatment for the 0.1 mg/kg/day dose group; and at additional posttreatment days for the 0.03 and 0.014 mg/kg/day dose groups. Plasma cholinesterase activity was measured. No effects were seen on plasma cholinesterase activity after 1 and 3 days of treatment. Plasma cholinesterase value was not significantly decreased at any dose level as compared to the baseline measurement. Significant inhibition of plasma cholinesterase activity was seen a this dose (0.1 mg/kg/day) only after 6 and 9 days of treatment. Mean values were 43% lower than baseline on 6 day and 64% lower than baseline on day 9 . The NOEL was 0.03 mg/kg/day and the LOEL was 0.10 mg/kg/day.

Dose/Endpoint for establishing the RfD: NOEL = 0.03 mg/kg/day based on statistically significant decrease in plasma ChEI on days 6 and 9 after treatment at 0.10 mg/kg/day.

Comments about Study and Endpoint: None.

Uncertainty Factor (UF): 100 (see discussion below).

Revised RfD = $\frac{0.03 \text{ mg/kg/day (NOEL)}}{100 \text{ (UF)}}$ = **0.0003 mg/kg/day**

Reassessment of the RfD: The HIARC concurred with the dose, endpoint and the study used in 1988 but determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be retained.** Therefore, for chronic dietary risk assessment, a UF of 100 is required (10 x for intra-species variation and 10 x for FQPA). **Consequently the revised RfD is 0.0003 mg/kg/day.** The UF of 100 is based on the following weight-of-the evidence considerations:

1. Chlorpyrifos is a neurotoxicant with evidence of OPIDN in humans and animals; there have been cases reported of neurophysiological effects in humans (See *Appendices A - E).*

2. Although no increased susceptibility was seen in young rats or rabbits following *in utero* exposure or in pups as compared to adults in the two-generation reproduction study in rats in the Subdivision F Guideline studies, increased susceptibility of offspring to the effects of Chlorpyrifos exposure has been identified in studies conducted in various reputable scientific research laboratories and reported in the open literature (See (*Appendices A - E).*

3. There are data gaps for the assessment of functional development of young animals following pre- and/or postnatal Chlorpyrifos exposure and thus the need for a developmental neurotoxicity study.

4. There are a variety of uses for Chlorpyrifos that may involve exposure to infants and children.

C. Occupational/Residential Exposure Risk Assessments

1. Dermal Absorption

Study selected :- Pharmacokinetics in human volunteers following single oral and dermal doses

MIRD No.: 000249203

Executive Summary: Six human male subjects were orally dosed with 0.5 mg/kg of Chlorpyrifos. In addition, five subjects were dosed once dermally with 5 mg/kg of Chlorpyrifos. Plasma cholinesterase depression was observed by the oral route, but not by the dermal route. On the basis of urinary excretion of 3,5,6-trichloro-2-pyridinol metabolite, the minimum absorption orally is approximately 70% and dermally approximately 1%.

Dermal Absorption Factor: 1%

2. Short-Term Dermal - (1-7 days)

Study Selected: 28-day Oral Toxicity - Human

MRID No. 00112118

Executive Summary: See Acute Dietary

Dose and Endpoint for Risk Assessment: NOEL = 0.10 mg/kg/day based on lack of inhibition of plasma cholinesterase activity after 1 and 3 days of treatment.

Comments about Study and Endpoint: Since plasma ChEI was seen only after 6 and 9 days of treatment, this dose was considered to be the NOEL for this exposure period of concern (i.e., 1-7 days time period). Since an oral NOEL was selected, a dermal absorption factor of 1% should be used for risk assessment.

This risk assessment is required.

3. Intermediate-Term Dermal (7 Days to Several Months)

Study Selected: 28-day Oral Toxicity - Human

MRID No. 00112118

Executive Summary: See Acute Dietary

Dose and Endpoint for Risk Assessment: NOEL=0.03 mg/kg/day based on significant plasma ChEI after 6 and 9 days of treatment at 0.10 mg/kg/day (LOEL).

Comments about Study and Endpoint: Since an oral dose was identified, a dermal absorption rate of 1% should be used for dermal risk assessments.

This risk assessment is required.

4. Long-Term Dermal (Several Months to Life-Time)

Study Selected: 28-day Oral Toxicity - Human

MRID No. 00112118

Executive Summary: See Acute Dietary

Dose and Endpoint for Risk Assessment: NOEL=0.03 mg/kg/day based on significant plasma ChEI after 6 and 9 days of treatment at 0.10 mg/kg/day (LOEL).

Comments about Study and Endpoint: Since an oral dose was identified, a dermal absorption rate of 1% should be used for dermal risk assessments.

This risk assessment is required.

5. Inhalation Exposure (Any-Time period)

Study Selected: 90-day Inhalation Toxicity - Rat §82-4

MRID No(s). 40013901, 40166501, 40908401

Executive Summaries: In the first study (MRID Nos.40013901 & 40166501), Fischer 344 rats (10/sex/concentration) were exposed nose only to Chlorpyrifos at concentrations of 0, 5.2, 10.3, or 20.6 ppb (0, 72, 143 or 287 $\mu g/m^3$, respectively) 6 hours/day, 5 days/week for 13 weeks. The NOEL was >20 ppb (287 $\mu g/m^3$); a LOEL was not established.

In the second study (MRID No. 40908401), Fischer 344 rats (10/ sex/ concentration) were exposed nose only to Chlorpyrifos at concentrations of 0, 5, 10, or 20.6 ppb (0, 72, 143 or 287 $\mu g/m^3$, respectively) 6 hours/day, 5 days/week for 13 weeks. These systemic toxicity and cholinesterase enzyme NOELs also exceeded 20 ppb; a LOEL was not established.

The HIARC concluded that the weight of evidence indicates that a inhalation hazard from technical Chlorpyrifos is unlikely based on the following factors: 1) the low vapor pressure of Chlorpyrifos; 2) the maximum attainable aerosol concentration of Chlorpyrifos (25 ppb at 25°C); 3) the two 90 day inhalation studies (nose only) that yielded no adverse effects at concentrations up to 20 ppb.

However, the Committee concluded that there is a possibility of mixer/loader/applicator exposure to formulations of Chlorpyrifos. Available exposure evidence on the termiticide use suggests that there is no measurable airborne concentrations of Chlorpyrifos after two days. Therefore, the Committee recommended that the oral NOEL from the Human study be used, and the appropriate route to route extrapolations be done. The oral NOELs of 0.10 mg/kg/day for Short-Term and the oral NOEL of 0.03 mg/kg/day for Intermediate- and Long-Term exposure risk assessments. Since the oral NOELs are selected, inhalation risk assessments should be as follows:

(i) The inhalation exposure component (i.e., mg/L) using a 100 % absorption rate (default value) should be converted to an *equivalent oral dose* (mg/kg/day).

(ii) The dermal exposure component (i.e., mg/kg/day) using 1% dermal absorption should be combined with this *converted oral dose* (mg/kg/day).

(iii) This dose should then be compared to the oral NOEL of 0.10 mg/kg/day for Short-Term and 0.03 mg/kg/day for Intermediate-and Long-Term exposures to calculate the Margins of Exposure.

This risk assessment is required.

The Acute Inhalation Toxicity study (Accession No. 257590) is classified as Supplementary because only nominal concentrations were measured in this study. The LC_{50} was greater than 0.2 mg/L in rats, which is normally assigned Toxicity Category II). The HIARC concluded that an acute inhaltion study does not need to be repeated since the physical state of technical Chlorpyrifos is a waxy solid, and there was no apparent indication that a high vapor concentration could be reached to cause a significant degree of acute toxicity in humans. This conclusion is supported by: 1) The theoretical maximum vapor concentration of chlorpyrifos is 25 ppb at 25°C; and 2) The Merck Index (11th Edition) reports the vapor pressure of chlorpyrifos at25°C to be 1.87×10^{-5} mm Hg.

D. Margins of Exposure for Occupational/Residential Exposures:

For Short-,Intermediate-,and Long-Term dermal and Inhalation risk assessments, HIARC determined that **a MOE of 100 is required** and includes the conventional 10 x for using a human study and a 10 x for FQPA. The rational for retaining the 10 x factor to account for enhanced sensitivity of infants and children (as required by FQPA) are presented in Sections II.1 and 2 for Acute and 2 Chronic Dietary risk assessments, respectively.

E. Recommendations for Aggregate Exposure Risk Assessments

An oral NOEL was selected for calculating the MOE's from oral, dermal and inhalation exposures. The dermal and inhalation exposure, using appropriate absorption factors, are converted to oral equivalent doses. Therefore,

For **acute** aggregate exposure risk assessment, combine the high end exposure values from food + water and compare it to the oral NOEL to calculate the MOE.

For **short, intermediate and chronic** aggregate exposure risk assessment, combine the average exposure values from food + water together with the aggregate exposures from dermal + inhalation and compare it to the oral NOEL to calculate the MOE.

III. FQPA CONSIDERATIONS

1. Neurotoxicity Data

The neurotoxicity profile of Chlorpyrifos was examined at a Chlorpyrifos Risk Dialogue Panel held in Chapel Hill, NC (2/8/96). The panel, commissioned by the Registrant, DowElanco, was comprised of experts from academia, industry, government, and risk assessment consulting. A session on developmental neurotoxicity was chaired by Stephanie Padilla (USEPA/NHEERL) and produced the following recommendations for further study:

(i) More completely evaluate the data base supporting the enhanced susceptibility of young animals to Chlorpyrifos and evaluate alterations in kinetic constants in young animals.

(ii) Conduct a standard developmental neurotoxicity screening test with Chlorpyrifos including measurement of blood and brain AChE activities.

(iii) Conduct a second screening study covering postnatal days 10-21 and include measurements of brain stem auditory evoked responses and protein/DNA synthesis in the brain.

(iv) Develop a pharmacokinetic model for Chlorpyrifos in the developing animal.

(v) For reported human studies, conduct a retrospective analysis of available cases and consider a prospective study in highly-exposed populations.

(vi) For hypothesis driven research, evaluate the development of AChE fibers in the brain and map the muscarinic and nicotinic receptors in the brain.

(vii) Consider the need to more adequately address the aging/aged animal model for Chlorpyrifos sensitivity.

The RfD Peer Review Committee in the previous meeting agreed with the recommendations of the Chlorpyrifos Risk Dialogue Panel, recognizing that a study in which neonates are directly exposed postnatally will be critical to the hazard assessment of Chlorpyrifos under FQPA.

2. Determination of Susceptibility

A review of the data that were submitted to the Agency for the reregistration of Chlorpyrifos, as well as numerous published and unpublished research papers, provided sufficient information to elicit concern about the potential for increased susceptibility of neonates to Chlorpyrifos exposure. On the other hand, there is no indication of increased susceptibility of fetuses to Chlorpyrifos following *in utero* exposure. The studies from which these conclusions are derived are described below, and the data that support these statements are presented in *Appendices B-E.*

(i) Postnatal exposure protocols

- Several multigeneration reproduction studies in rats (§83-4) were conducted with Chlorpyrifos (Thompson, et al. 1971; Dietz et al., 1983; James, 1989; Breslin, 1991). In none of these studies was an increased susceptibility of the offspring demonstrated following *in utero* or postnatal exposure.

- Studies from the open literature, specifically addressed the differences between the response of adult rats versus neonatal or weanling rats following either acute or subacute doses of Chlorpyrifos. In all of these studies, the Chlorpyrifos was administered by subcutaneous injection which does not approximate any exact scenario of potential human exposure, although it could be argued that it may mimic some aspects of direct dermal exposure to Chlorpyrifos.

  It was demonstrated that neonatal rats were much more sensitive to acute doses of Chlorpyrifos at levels near the maximum tolerated dose than were adult rats, as measured by lethality (LD10 values) (Pope *et al.*, 1991). However, measurement of neurobiochemical and/or neurobehavioral endpoints demonstrated that when Chlorpyrifos was administered postnatally by repeated subtoxic doses, adult rats were more sensitive than neonatal or weanling rats (Pope and Chakraborti, 1992; Chakraborti *et al.*, 1993; Stanton *et al.*, 1994).

- In a 1996 study by Moser and Padilla, neonatal rats (10-27 days of age) were found to be between 5-7 times more sensitive than adults to doses of Chlorpyrifos administered orally at the maximum tolerated dose. Neurobehavioral measurements following oral administration of Chlorpyrifos (80 mg/kg for adults and 15 mg/kg for pups) demonstrate that young rats respond with similar behavior changes as adults, but at a 5-fold lower dose.

- In a study by Whitney *et al.* (1995) Chlorpyrifos (2 mg/kg) was administered by subcutaneous injection to rat neonates (postnatal day 1). Within 4 hours, significant inhibition of DNA and protein synthesis was observed in the cerebellum, forebrain, and brainstem. This work has reportedly been repeated and confirmed by Slotkin et al. (1997). Although it is intuitively reasoned that such disruption in brain development could have long-lasting neurological and/or neurobehavioral effects, particularly after repeated or prolonged subtoxic exposures, no studies have been completed that address these issues directly. Preliminary reports of an NIEHS study on Chlorpyrifos, which has recently been completed did not identify neurobehavioral effects in neonatal or weanling rats that were exposed pre- and postnatally.

(ii). *In utero* exposure protocols

- Numerous prenatal developmental toxicity studies (§83-3) have been conducted with Chlorpyrifos (Deacon *et al.*, 1979 and 1980; Oullette *et al.*, 1983; Rubin *et al.*, 1987a; Rubin *et al.*, 1987b; Thompson, 1971). Regardless of species or strain, none have demonstrated evidence of susceptibility of the offspring following *in utero* exposure.

- Studies were conducted which specifically addressed the differences between the response of maternal versus fetal or neonatal rats following either acute or subacute doses of Chlorpyrifos by subcutaneous injection during gestation. Measurement of neuro-biochemical and/or neurobehavioral endpoints demonstrated that when Chlorpyrifos was administered during gestation, maternal rats were more sensitive to Chlorpyrifos exposure than were their fetuses or neonates (Chanda et al., 1995; Chanda and Pope, 1996; Barone et al., 1997; Phillips et al., 1997; Lassiter et al., 1997a).. This protective effect was not found to be due to placental metabolism of Chlorpyrifos (Lassiter et al, 1997b).

3. Recommendation for a Developmental Neurotoxicity Study

The RfD Peer Review Committee (9/14/93) determined that an assessment of the potential for developmental neurotoxicity was required because of the evidence of adult and fetal ChE inhibition, and the suggestion of cognitive effects in adult animals.

4. Adequacy of Data Package

An acceptable two-generation reproduction study in rats and acceptable prenatal developmental toxicity studies in rats, mice, and rabbits have been submitted to the Agency, meeting the minimal core data requirements for a food-use chemical, as defined by 40 CFR Part 158.

5. Determination of Uncertainty Factor

The HIARC determined that the 10 x factor for enhanced sensitivity of infants and children (as required by FQPA) should be retained based on the following weight-of-the-evidence considerations::

(i) Chlorpyrifos is a neurotoxicant with evidence of OPIDN in humans and animals; there have been cases reported of neurophysiological effects in humans.

(ii) Increased susceptibility of offspring to the effects of Chlorpyrifos exposure has been identified in studies conducted in various reputable scientific research laboratories and reported in the open literature.

(iii) There are data gaps for the assessment of functional development of young animals following pre- and/or postnatal Chlorpyrifos exposure and thus the need for a developmental neurotoxicity study.

(iv) There are a variety of uses for Chlorpyrifos that may involve exposure to infants and children.

IV. REVIEW OF REGISTRANT'S REFERENCE DOSE PROPOSAL

The Registrant submitted a comprehensive and succinct review of a wide variety of toxicology studies on Chlorpyrifos (MRID No. 44271001). This report was itself reviewed and endorsed by an external peer review committee convened by the Registrant. Reference doses for acute and chronic exposures to Chlorpyrifos were proposed based on its analysis of the appropriate NOELs and uncertainty factors based on its own analyses of the studies and the criteria described by the Acute Cholinesterase Risk Assessment Task Force (ACRA).

This review also addresses some of the studies on the increased sensitivity of neonatal rats in comparison to adult rats. In brief, their review does not refute support for the idea that neonatal rats are more sensitive than adult rats to the lethal effects of Chlorpyrifos. Nor does it discuss the more recent data from EPA (Moser and Padilla, 1997) on this issue. While the Registrant (DowElanco) here maintains that differences in rates of maturation appear to invalidate extrapolation to humans, the logic of this argument is not immediately apparent, and this is the only kind of data (from animals) that we are likely to have on which to base these findings.

In addition, this report reviews and makes comment on a variety of other studies submitted to EPA or in the open literature, with which EPA has different opinions. This brief analysis has focussed on those relevant to the hazard identification report and its conclusions.

For acute dietary risk assessment, the TES Committee determined a NOEL of 0.10 mg/kg from the lack of any effects in the first days of the 28-day human study (Coulston *et al*). The Registrant, however, found the NOEL for acute effects to be 0.5 mg/kg, based on another human study (Nolan *et al.*) in which they concluded that no significant effects were seen at that dose. In the Agency's presentation to the FIFRA Scientific Advisory Panel (SA) meeting in June 1997, the Agency noted that in this study, on Day 1, plasma cholinesterasse acivity was inhibited 84-86%, while by Day 4 after the dose, RBC acetylcholinesterase activity was inhibited 11-52%. Therefore, the HIARC rejected the notion of 0.5 mg/kg/day as a NOEL for *either* plasma or RBC cholinesterase inhibtion from the Nolan *et al.* study.

For chronic dietary risk assessment (i.e, for Rfd), both HED and the Registrant, focused on the same human study (Coulston et al.). HED's alternative interpretations of these data are the same as previously discussed and debated. Therefore, the Committee re-affirmed the NOEL to be 0.03 mg/kg for deriving the RfD.

The NOELs established in the human study were also used for Short-, Intermediate-and Long-Term dermal and inhalation occupational/residential exposure risk assessments. No alternatives were suggested by the Registrant in this report.

The author of the Registrant's report, Nolan, also derives NOELs for acute and repeated exposures in animals. He cites an acute rat NOEL for RBC and plasma ChE as 1 mg/kg. Since effects were found in the acute oral human study at 0.5 mg/kg, this takes precedence over the rat data cited. Finally, the repeated exposure NOEL cited by Nolan is 1 mg/kg. However, a number of studies cited by EPA in its earlier RfD report (1993) note NOELs less than 1 mg/kg. In the carcinogenicity study with rats (MRID 42172801), the NOEL was considered less than 0.0132 mg/kg for females for ChE inhibition. In the chronic toxicity study with rats (MRID 40952802), HED considers the NOEL for brain and plasma ChEI to be 0.1 mg/kg .

For the data directly related to the RfD or other toxicological endpoints used for current human risk assessments, no new data or analyses were provided in this report that had not previously been considered by HED's RfD Committee in earlier meetings. The RfD Peer Review Report of 2/29/96 discussed the same data from the Registrant in terms of acute and repeated animal and human exposures.

Since the weight-of-the-evidence approach to application of uncertainty factors for cholinesterase inhibition data used by OPP and applied to Chlorpyrifos was recently approved by the SAP, which also considered the alternatives proposed by ACRA, there is no further need to address the application of these alternative criteria for Chlorpyrifos.

After careful consideration of the animal toxicology and available human data as well as the proposals put forth by the Registrant, the Committee concluded that there is no reason for changing the previous doses and end points selected for human risk assessments. Therefore, the HIARC re-affirmed the doses and endpoints selected for acute and chronic dietary as well as the occupational/residential exposure risk assessments. A summary of the toxicology endpoints selected are provided below:

V. SUMMARY OF TOXICOLOGY ENDPOINTS SELECTION

The doses and toxicological endpoints selected and Margins of Exposures for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	MOE
Acute Dietary	NOEL=0.10	Lack of acute effects at 1 and 3 days after treatment.	28-day oral-Human	100
Chronic Dietary	NOEL=0.03	Plasma cholinesterase inhibition on days 6 and 9.	28-day oral-Human	100
	Revised RfD = 0.0003 mg/kg/day			
Short-Term (Dermal)	Oral NOEL =0.10	Lack of effects upto day 7.	28-day oral-Human	100
Intermediate-Term (Dermal)	Oral NOEL=0.03	Plasma cholinesterase inhibition on days 6 and 9.	28-day oral-Human	100
Long-Term (Dermal)	Oral NOEL=0.03	Plasma cholinesterase inhibition on days 6 and 9.	28-day oral- Human	100
Short-Term (Inhalation)	Oral NOEL=0.10	Lack of effects upto day 7.	28-day oral-Human	100
Intermediate-Term (Inhalation)	Oral NOEL=0.03	Plasma cholinesterase inhibition on days 6 and 9	28-day oral-Human	100
Long-Term (Inhalation)	Oral NOEL=0.03	Plasma cholinesterase inhibition on days 6 and 9	28 day oral-Human	100

APPENDIX A

Data Summaries: **Neurotoxicity Studies**

In addition to the toxicology data base available to the Agency, the RfD Committee searched the open literature in order to better address the issue of plasma and brain cholinesterase correlation. Open literature information reviewed by the Committee demonstrated that, in the rat, plasma cholinesterase inhibition has been observed to correlate very well with brain cholinesterase inhibition following the administration of single doses of certain organophosphorus pesticides. The following are excerpts taken from a review by Dr. Brian Dementi, HED, OPP for three open literature studies used by the RfD Peer Review Committee in partial support of its current position on issues raised by the registrant.

1 Pope and Chakraborti (1992) evaluated the effects of three organophosphorus pesticides; parathion, methyl parathion and Chlorpyrifos administered subcutaneously, in both adults and neonates SD rats. Good correlations were observed between ED_{50} values (dose inhibiting the enzyme 50%) and the Maximum Tolerated Dose (MTD), used as an indication of toxicity. In this publication the authors stated (P.41): "For example, when brain cholinesterase ED_{50} values were correlated with MTDs for both age groups, a correlation (r) value of 0.932 was obtained, indicating a good correlation between brain cholinesterase inhibitory potency and acute toxicity among the inhibitors. An even higher correlation (r=0.992) was noted, however, between plasma cholinesterase ED_{50} values and MTDs. In addition, there were no significant differences in the ED_{50} values of brain cholinesterase relative to plasma cholinesterase with either of the OP treatments in either age group." The authors indicated that, while plasma cholinesterase levels, under defined experimental conditions, may provide a quantitative estimate of the extent of cholinesterase inhibition in the central nervous system following organophosphate exposure, factors such as route of exposure and time after treatment when cholinesterase is assayed could influence the degree of correlation.

2. Pope et al (1992) investigated the effects of Chlorpyrifos in Sprague-Dawley rats following subcutaneous administration at the MTD, on a number of parameters, including plasma and brain cholinesterase inhibition SD rats. Following 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.

In any case, there is no reason to conclude that brain cholinesterase inhibition in the human case study would not be a correlate of plasma cholinesterase inhibition.

Appendix B

Data Summaries: **Prenatal Developmental Toxicity Studies**

The following prenatal developmental toxicity studies (§83-3) have been conducted with Chlorpyrifos. None have demonstrated evidence of susceptibility of the offspring following *in utero* exposure. The studies by Deacon et al. (1979), Oulette et al. (1983), Rubin et al. (1987a) and Rubin et al. (1987b) were found to be acceptable by GLP and guideline (§83-3) standards and were considered in support of the registration of Chlorpyrifos.

1. In a developmental toxicity study in two coherts of CF-1 mice, Chlorpyrifos was administered by gavage at doses of 0.1, 1, 10, or 25 mg/kg/day, on gestation days 6-15. Fetotoxicity was noted at 25 mg/kg/day, a dose at which severe maternal clinical toxicity was also observed. The developmental and maternal systemic NOELs were both 10 mg/kg/day. Maternal plasma cholinesterase depression was noted at 1 mg/kg/day (maternal ChE NOEL = 0.1 mg/kg/day), while fetal homogenate cholinesterase levels were significantly depressed at 10 mg/kg/day and greater. (MRID 00095268; Deacon et al., 1979; also reported in Deacon et al., 1980)

2. In a study in Fischer 344 rats, Chlorpyrifos was administered by gavage on gestation days 6-15, at dose levels of 0.1, 3.0, or 15.0 mg/kg/day. No signs of developmental toxicity were observed at doses up to and including 15. mg/kg/day, although maternal toxicity was noted at 3 mg/kg/day (plasma and RBC cholinesterase inhibition) and greater. (MRID 00130400; Oullette et al., 1983; also reported in Breslin et al., 1996)

3. Chlorpyrifos was administered to Sprague-Dawley rats on days 6-15 of gestation, at gavage doses of 0.5, 2.5, or 15.0 mg/kg/day. Plasma cholinesterase inhibition was noted in the dams at 0.5 mg/kg/day; therefore, a maternal ChE NOEL was not identified. Maternal systemic effects (decreased body weight and food consumption) were observed at 2.5 mg/kg/day, with a maternal systemic NOEL at 0.5 mg/kg/day. Increased postimplantation loss was observed at 15.0 mg/kg/day; the developmental NOEL was 2.5 mg/kg/day. (MRID 40436407; Rubin et al., 1987a)

4. In a prenatal developmental toxicity study in Himalayan rabbits, Chlorpyrifos was administered by gavage on gestation days 7-19, at doses of 1, 9, 81, or 140 mg/kg/day. Maternal weight gain was depressed during the treatment period at 140 mg/kg/day (maternal systemic NOEL = 81 mg/kg/day), and plasma cholinesterase was inhibited at all dose levels (the maternal ChE NOEL was not determined). Slight decreases in fetal weight and length, and increased incidences of skeletal variations and ossification delays in the sternebrae were observed at 140 mg/kg/day, with a developmental NOEL of 81 mg/kg/day. (MRID 40436408; Rubin et al., 1987b)

5. In a developmental toxicology study, conducted as a segment of a dietary three-generation reproduction study, dietary concentrations of Chlorpyrifos were administered at levels of 0.1, 0.3, and 1.0 mg/kg/day to F2b dams. No treatment-related effects on F3b fetuses were demonstrated at dose up to and including the highest dose tested (1.0 mg/kg/day), a dose at which plasma and RBC cholinesterase were decreased in the adults. (MRID 00029064, 00064934; Thompson, 1971)

Appendix C
Data Summaries: **Multigeneration Reproduction Studies**

Several multigeneration reproduction studies in rats (§83-4) were conducted with Chlorpyrifos. In none of these studies was an increased susceptibility of the offspring demonstrated following *in utero* or postnatal exposure. The study by Breslin (1991) was found to be acceptable by GLP and guideline (§83-4) standards and was considered in support of the registration of Chlorpyrifos.

1. In a three-generation reproduction study in Sprague-Dawley rats, dietary concentrations of Chlorpyrifos were administered at levels of 0.03, 0.1, or 0.3 mg/kg/day in the first generation and at 0.1, 0.3, and 1.0 mg/kg/day thereafter. No toxicological effects were noted in the offspring (cholinesterase levels were not measured in pups). There was a decrease in plasma and RBC cholinesterase activity at 0.3 and 1.0 mg/kg/day in the parental males and females (ChE NOEL = 0.1 mg/kg/day); no other evidence of parental systemic toxicity was observed. (MRID 00029064, 00064934; Thompson et al., 1971)

2. A two-generation reproduction study in Sprague-Dawley rats was conducted to establish a NOEL for neonatal survival. Chlorpyrifos was administered in the diet at doses of 0.5, 0.8, or 1.2 mg/kg/day. No evidence of treatment-related toxicity was observed in the offspring, while systemic toxicity in the adults (decreased body weight gain in males) was noted at 1.2 mg/kg/day (systemic NOEL = 0.8 mg/kg/day). (MRID 00130401; Dietz et al., 1983)

3. In a two-generation reproduction study in rats, Chlorpyrifos was administered at dietary levels of 2, 10, or 50 ppm (equivalent to 0.13/0.14, 0.64/0.71, or 3.21/3.58 mg/kg/day in F0 M/F) no systemic effects were observed in either the parental animals or the offspring at dietary levels up to and including 50 ppm. However, in a range-finding study that was used to select treatment levels for the two-generation reproduction study, dietary levels of 50 ppm resulted in plasma, RBC, and brain cholinesterase inhibition in adult females, and in plasma and RBC (but not brain) cholinesterase inhibition in male and female weanlings. (MRID 42172803; James, 1989)

4. In a two-generation reproduction study in Sprague-Dawley rats, Chlorpyrifos was administered in the diet at levels of 0.1, 1.0, or 5.0 mg/kg/day. Offspring toxicity consisted of reduced pup body weight and increased postnatal mortality at 5.0 mg/kg/day. In the adult animals of both generations, at a dose of 1.0 mg/kg/day, decreased plasma and RBC cholinesterase activity were observed in both sexes, and histopathological lesions of the adrenal gland were observed in the females. Additionally, at 5.0 mg/kg/day, brain cholinesterase was decreased in males and females of both generations. Although cholinesterase activity was not assessed in weanlings, the NOEL for toxicity in the offspring (1.0 mg/kg/day) was greater than the adult systemic NOEL of 0.1 mg/kg/day. (MRID 41930301; Breslin, 1991).

Appendix D

Data Summaries: **Studies from the Open Literature**

In a review of the open literature, studies were identified which specifically addressed the differences between the response of adult rats versus fetal, neonatal, or weanling rats following either acute or subacute doses of Chlorpyrifos. In all of these studies, the Chlorpyrifos was administered by subcutaneous injection which does not approximate any exact scenario of potential human exposure, although it could be argued that it may mimic some aspects of direct dermal exposure to Chlorpyrifos. Nevertheless, the conclusions of these studies support the findings of the guideline studies, as described above. In summary, neonatal rats were shown to be much more sensitive to acute doses of Chlorpyrifos at levels near the maximum tolerated dose than are adult rats, as measured by lethality (LD10 values). However, measurement of neurobiochemical and/or neurobehavioral endpoints demonstrated that when Chlorpyrifos was administered during gestation, maternal rats were more sensitive to Chlorpyrifos exposure than were their fetuses or neonates, and when Chlorpyrifos was administered postnatally, adult rats were more sensitive than neonatal or weanling rats.

A. Studies that address the neurochemical and neurobehavioral effects of maternal versus fetal or neonatal rats following *in utero* exposure to Chlorpyrifos are described below:

1. In a study by Chanda *et al.* (1995), which expanded findings reported by Chanda et al. in 1993, a single dose of 200 mg/kg of Chlorpyrifos was administered by subcutaneous injection to Sprague-Dawley rats on gestation day 12. Any dams with moderate to severe signs of cholinergic toxicity at 2-3 days after dosing were eliminated from study. The dams that remained on study and their offspring were killed on gestation day 16 or 20 or postnatal day 3 for tissue collection and analysis. Extensive AChE inhibition (82-88%) was noted in maternal brain at all three time points following acute exposure. At gestation days 16 and 20, fetal brain AChE activity was inhibited 42-44%. While some degree of recovery was observed in pup brain by postnatal day 3, AChE activity was still inhibited 30% in treated pups cross-fostered to control dams. In vitro inhibition of maternal and fetal (gestation day 20) brain AChE activity by the active metabolite, Chlorpyrifos oxon, suggested that the prenatal brain AChE activity was somewhat more sensitive (IC50 at 37.0°C, 20 min. dam, 26.6 $\pm$ 1.8 x 10^{-9} M; fetus, 6.7 $\pm$ 0.4 x 10^{-9} M). Maternal brain muscarinic receptor binding was more extensively reduced (30-32%) at gestation day 20 and postnatal day 3 as compared to the developing brain at gestation day 20 (16%) and postnatal day 3 (11%). A simple postnatal reflex test, righting reflex, was transiently altered by Chlorpyrifos. The study authors 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 (80-100 days of age) Sprague-Dawley rats after acute treatment (by subcutaneous injection) with maximum tolerated doses of Chlorpyrifos

and other organophosphate pesticides. The neonates were more sensitive clinically than adults to Chlorpyrifos exposure: the MTD for neonates was 45 mg/kg s.c., while for adults the MTD was 279 mg/kg s.c. In general, maximal brain ChE inhibition was similar (>78%) in both age groups, but ChE activity recovered faster in neonates. Plasma and RBC ChE activities correlated relatively well with brain ChE activity in neonatal rats at all time points between 4 hours and 7 days posttreatment, but similar correlations between circulating and brain ChE activities in adults were more variable. The study authors concluded that neonatal rats are more sensitive to acute lethality from Chlorpyrifos (and other OP) exposure than are adults, and that MTD exposures produced extensive brain ChE inhibition in both age groups. Following OP exposures, however, significant compound-related and age-related differences in the duration of ChE inhibition can occur.

2. In a paper published in 1992, Pope and Chakraborti described a study in which they examined dose-related inhibition of both brain and plasma cholinesterase activity in neonatal and adult rats exposed to Chlorpyrifos and other organophosphate pesticides. It was found that ED_{50} estimates for both brain and plasma cholinesterase correlated highly with previously derived MTD values. The correlation between the extent of brain and plasma cholinesterase inhibition across dose in neonatal rats was high but lower in adults. The study authors concluded that in vivo inhibitory potency of Chlorpyrifos and the other organophosphate pesticides tested towards either brain or plasma ChE activity is highly correlated with sensitivity to acute toxicity in both neonatal and adult rats.

3. Chakraborti *et al.* (1993) further pursued the premise that neonatal rat (7 days of age) are markedly more sensitive to acute toxic effects of Chlorpyrifos exposure than are adult rats (3 months of age), and compared subacute exposures in the same age groups. Repeated doses of Chlorpyrifos (40 mg/kg by subcutaneous injection, every 4 days for a total of 4 doses) resulted in extensive inhibition of cortical hippocampal, and striatal cholinesterase activity in adult Sprague-Dawley rats at 4 (90-92%) and 14 (71-78%) days after the last treatment. Young rats treated in the same manner, beginning of postnatal day 7, showed a much lower degree of ChE inhibition (21-60%) at these time points. Muscarinic receptor ([^{3}H]quinuclidinyl benzilate, QNB) binding in cortex, hippocampus, and striatum was reduced in adult brain at 4 (30-43%) and 14 (22-32%) days after the final treatment, whereas receptor densities were only marginally affected in young rats (5-11% reduction). Basal motor activity levels were 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 also suggests that the Chlorpyrifos does not need to be

metabolically activated to the oxon in order to produce neurological effects in neonates. At 8 days of age, inhibition of DNA synthesis was also seen; however, there was regional selectivity, with sparing of the cerebellum. It was also determined that the effects of Chlorpyrifos on DNA and protein synthesis were not secondary to generalized cell damage or suppression of cell metabolism, since ornithine decarboxylase activities were normal. The study authors concluded that low doses of Chlorpyrifos target the developing brain during the critical period in which cell division is occurring, effects which may produce eventual cellular, synaptic, and behavioral aberrations after repeated or prolonged subtoxic exposures.

Appendix E

Data Summaries: **Additional Studies which are in Preparation for Publication**

Additional studies have been identified which have not been published in the peer-reviewed literature, but which are in process of preparation for publication. In these studies, the issues of differential sensitivities between adults and young animals following *in utero* and/or postnatal exposure to Chlorpyrifos were further addressed. A major difference between these and previous studies was that exposure was by the oral route, rather than by subcutaneous injection.

A. Studies that address the neurochemical and neurobehavioral effects of maternal versus fetal or neonatal rats following *in utero* exposure to Chlorpyrifos are described below:

1. Lassiter *et al.* conducted a study to compare the degree and define the time course of ChE inhibition in the dam, placenta, and fetus following late gestational exposure to Chlorpyrifos. The Chlorpyrifos was administered to Long Evans rats by gavage in corn oil at doses of 0 or 7 mg/kg/day on gestation days 14-18; animals were killed at 2, 5, 10, 24, 48, and 120 hours after the last dose. Body weight gain was not affected in treated dams, but maternal blood and brain ChE activity was inhibited (80-90%), reaching a nadir at 5 hours after the last dose. By 120 hours AChE activity had recovered to 30-45% inhibition. Fetal brain ChE inhibition was maximal at 25%, also at 5 hours after last dose, and recovered to control levels by 48 hours. To test the hypothesis that the placenta may be protecting the fetus from CPF-oxon, the placental tissue was analyzed. It was found that two of the enzymes known to detoxify CPF-oxon were either not enriched (Chlorpyrifos-oxonase) or only slightly enriched (carboxylesterase; $\leq$35%) in placental tissue as compared to maternal blood, indicating that the placenta may not be a site for preferential Chlorpyrifos-oxon metabolism which would protect the fetus.

2. In a study by Barone *et al.*, Chlorpyrifos was administered by gavage to Long Evans rats (16/group) on gestation days 14-18 at dose levels of 0, 3, or 5 mg/kg/day. A subset of animals was killed on gestation day 19; 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 activity were not used as an index of Chlorpyrifos-induced inhibition, but rather as molecular markers of normal brain development. In this study, Long Evans rats were administered Chlorpyrifos at 0, 3, or 5 mg/kg/day on gestation days 14-18. Brain regions were collected on postnatal days 1, 4, 7, 12, 17, 21, and 92. Cortical and cerebellar developmental profiles were studied because they represent early and late-developing regions, respectively. The developmental pattern of

AChE and BuChE activity varied with respect to brain region and age, but gestational Chlorpyrifos exposure appeared to have no effect on the region-specific profiles of either AChE or BuChE activity.

B. Studies that address the comparison of the neurotoxic response of adults and neonatal or weanling animals including possible mechanisms of the differences observed, are described below:

1. Moser and Padilla (1998) compared the effects of acute oral Chlorpyrifos exposure in adult (70 days of age) and young (postnatal day 17) rats. They verified the findings of Pope, that neonatal rats (10-27 days of age) were between 5-7 times more sensitive than adults to acute doses of Chlorpyrifos at the maximum tolerated dose, with greater sensitivity identified in the youngest neonates. Then the time-course of the effects of an acute dose of Chlorpyrifos was evaluated for adults and postnatal day 17 pups. Assessments included behavioral evaluations (functional observation battery and motor activity), cholinesterase activity measurements, and muscarinic receptor assays. Doses were administered by gavage at levels that were selected to produce similar effects in young and adult rats; adults received 80 mg/kg and pups received 15 mg/kg. Following testing, tissues were taken at 1, 2, 3.5, 6.5, 24, 72, 168, or 336 hours posttreatment. In adult rats, behavioral changes and brain and blood ChE inhibition followed the same temporal pattern. Peak effect occurred in male rats about 3.5 hours postdose. The onset of changes was quicker in females, but the time-course was more protracted and recovery was slower. In pups, maximal behavioral effects occurred 6.5 hours after dosing, without gender differences. Partial to full recovery of behavioral changes was observed at 24 and 72 hours, similar to adults. Blood and brain ChE inhibition in young rats had nearly recovered by 1 week postdose, but adult brain ChE had not fully recovered at 2 weeks. Muscarinic receptor binding assays showed apparent down-regulation in some brain areas at 24 and 72 hours. The study authors concluded that: 1) young rats show similar behavioral changes, although at a 5-fold lower dose; 2) the onset of maximal effects is somewhat delayed in the young rats, 3) ChE activity tends to recover more quickly in young rats, but; 4) the young rats appear to have more extensive muscarinic receptor down-regulation; and 5) young rats show no gender-related differences.

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 1/2, 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 insecticides were not different.

Literature references:

Andersen, M. (1996) A final report of the: Chlorpyrifos Neurotoxicity Workshop and the Chlorpyrifos Risk Dialogue Panel held at the Carolina Inn, Chapel Hill, NC, February 6-8, 1996. ICF Kaiser Engineers, Inc., RTP, NC.

Ashry, K.M., F.R. Ali, Y.A. Hussein, S.M. Hamza, and M.B. Abou-Donia. (1994) Inhibition of total and individual molecular forms of acetylcholinesterase (AChE) activity in pregnant rats and fetuses following a single oral dose of chlorpyrifos [abstract 910]. *Toxicologist* 14(1):242.

Barone, S., C. Lau, V.C. Moser, P.M. Phillips, K.L. McDaniel, D. Hunter, R. Marshall, P. Kodavanti, F. Dern-Yellin, and S. Padilla. (1997) Developmental effects of gestational exposure to chlorpyrifos in the rat [abstract 1301]. *Toxicologist* 36(1):256.

Breslin, W.J., A.B. Liberacki, D.A. Dittenber, and J.F. Quast. (1996) Evaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat. *Fundamental and Applied Toxicology* 29:119-130.

Campbell, C.G., Seidler, F.J, and Slotkin, T.A. (1997). Chlorpyrifos interferes with cell development in rat brain regions (Brain Res. Bull 43(2):179-189.

Chakraborti, T.K., J.D. Farrar, and C.N. Pope. (1993) Comparative neurochemical and neurobehavioral effects of repeated chlorpyrifos exposures in young and adult rats. *Pharmacology Biochemistry and Behavior* 46:219-224.

Chanda, S.M., J. Chaudhuri, T. Chakraborti, and C. Pope. (1993) Persistent fetal brain cholinesterase inhibition induced by a single maternal dose of chlorpyrifos [abstract 257]. *Toxicologist* 13:88.

Chanda, S.M., P. Harp, J. Liu, and C.N. Pope. (1995) Comparative developmental and maternal neurotoxicity following acute gestational exposure to chlorpyrifos in rats. *Journal of Toxicology and Environmental Health* 44:189-202.

Chanda, S.M., S.R. Mortensen, S. Barone, V.C. Moser, and S. Padilla. (1997) Developmental profiles of two organophosphate detoxifying enzymes: carboxylesterase and A-esterase [abstract 1757]. *Toxicologist* 36(1):346.

Chanda S.M. and C.N. Pope. (1996) Neurochemical and neurobehavioral effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. *Pharmacology Biochemistry and Behavior* 53(4):771-776.

Deacon, M.M., J.S. Murray, M.K. Pilny, K.S. Rao, D. A. Dittenber, T.R. Hanley, and J.A. John. (1980) Embryotoxicity and fetotoxicity of orally administered chlorpyrifos in mice. *Toxicology and Applied Pharmacology* 54:31-40.

Lassiter, T.L., S. Padilla, and S. Barone. (1997) Effects of gestational exposure to chlorpyrifos on the developmental profiles of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity in the rat brain [abstract 1313]. *Toxicologist* 36(1):259.

Lassiter, T.L., D. Hunter, R. Marshall, S. Mortensen, S. Chanda, K. Das, and S. Padilla. (1997) The fetal brain appears to be protected from late gestational exposure to chlorpyrifos. Platform/Poster submission, Neurobehavioral Teratology Society Meeting.

Mortensen, S.R., S.M. Chanda, M.J. Hooper, and S. Padilla. (1997, draft) Maturational differences in chlorpyrifos-oxonase activity may contribute to age-related sensitivity to chlorpyrifos.

Mortensen, S.R., M.J. Hooper, and S. Padilla. (1997, draft) Developmental profiles and maturational sensitivity of rat brain acetylcholinesterase activity.

Moser, V.C. and S. Padilla. (1998, draft) Age- and gender-related differences in the time-course of behavioral and biochemical effects produced by oral chlorpyrifos in rats. Submitted for publication to *Toxicology and Applied Pharmacology*.

Padilla S., Wilson, V.Z., and Bushnell, P.J. (1994). Studies on the correlation between blood cholinesterase inhibition and "Target Tissue" inhibition in pesticide-treated rats. Toxicology 92;11-25.

Phillips, P.M., K.L. McDaniel, T.L. Lassiter, S. Barone, and V.C. Moser. (1997) Behavioral effects of gestational exposure to chlorpyrifos in rats [abstract 1300]. *Toxicologist* 36(1):256.

Pope, C.N., T.K. Chakraborti, M.L. Chapman, J.D. Farrar and D. Arthun. (1991) Comparison of in vivo cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. *Toxicology* 68:51-61.

Pope, C.N. and T.K. Chakraborti. (1992) Dose-related inhibition of brain and plasma cholinesterase in neonatal and adult rats following sublethal organophosphate exposures. *Toxicology* 73:35-43.

Pope, C.N, Chakraborti, T.K., Chapman, M.L and Farrar, J.D. (1992). Long-Term neurobehavioral and behavioral effects induced by acute chlorpyrifos treatment (1992) Pharm.Biochem.Behav. 42:251-256

Stanton, M.E., W.R. Mundy, T. Ward, V. Dulchinos, and C.C. Barry. (1994) Time-dependent effects of acute chlorpyrifos administration on spatial delayed alternation and cholinergic neurochemistry in weanling rats. *NeuroToxicology* 15(1):201-208.