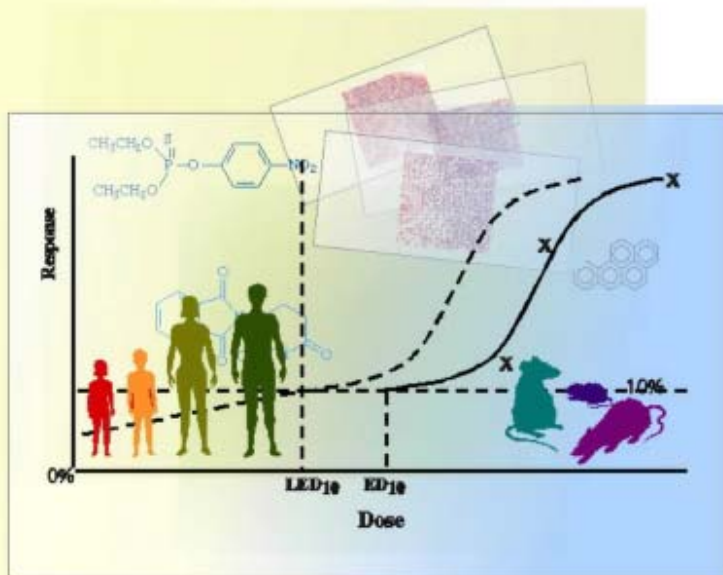


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Science Issue Paper: Chlorpyrifos Hazard and Dose Response Characterization

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1.0 Introduction

Chlorpyrifos (0,0-diethyl-0-3,5,6-trichloro -2-pyridyl phosphorothioate) is a broad-spectrum, chlorinated organophosphate (OP) insecticide. In 2000, nearly all residential uses were voluntarily cancelled by Dow AgroSciences but agricultural use remains. The 2000 human health risk assessment was largely based on adult laboratory animal data (rat or dog) for cholinesterase inhibition and the application of default uncertainty factors. Since 2000, there has been extensive research on various aspects of chlorpyrifos including its neurological effects in animals and humans following gestational and post-natal exposures, its pharmacokinetics, and mechanism of action. Additional, there are currently several regulatory efforts on-going for chlorpyrifos which have led EPA to update the hazard assessment and hazard characterization of chlorpyrifos. These efforts include the review of chlorpyrifos under the Pesticide Registration Improvement Act (PRIA) and registration review. In addition, the Natural Resources Defense Council (NRDC) and the Pesticide Action Network North America (PANNA) have petitioned the Agency to revoke all tolerances and cancel all uses of chlorpyrifos¹.

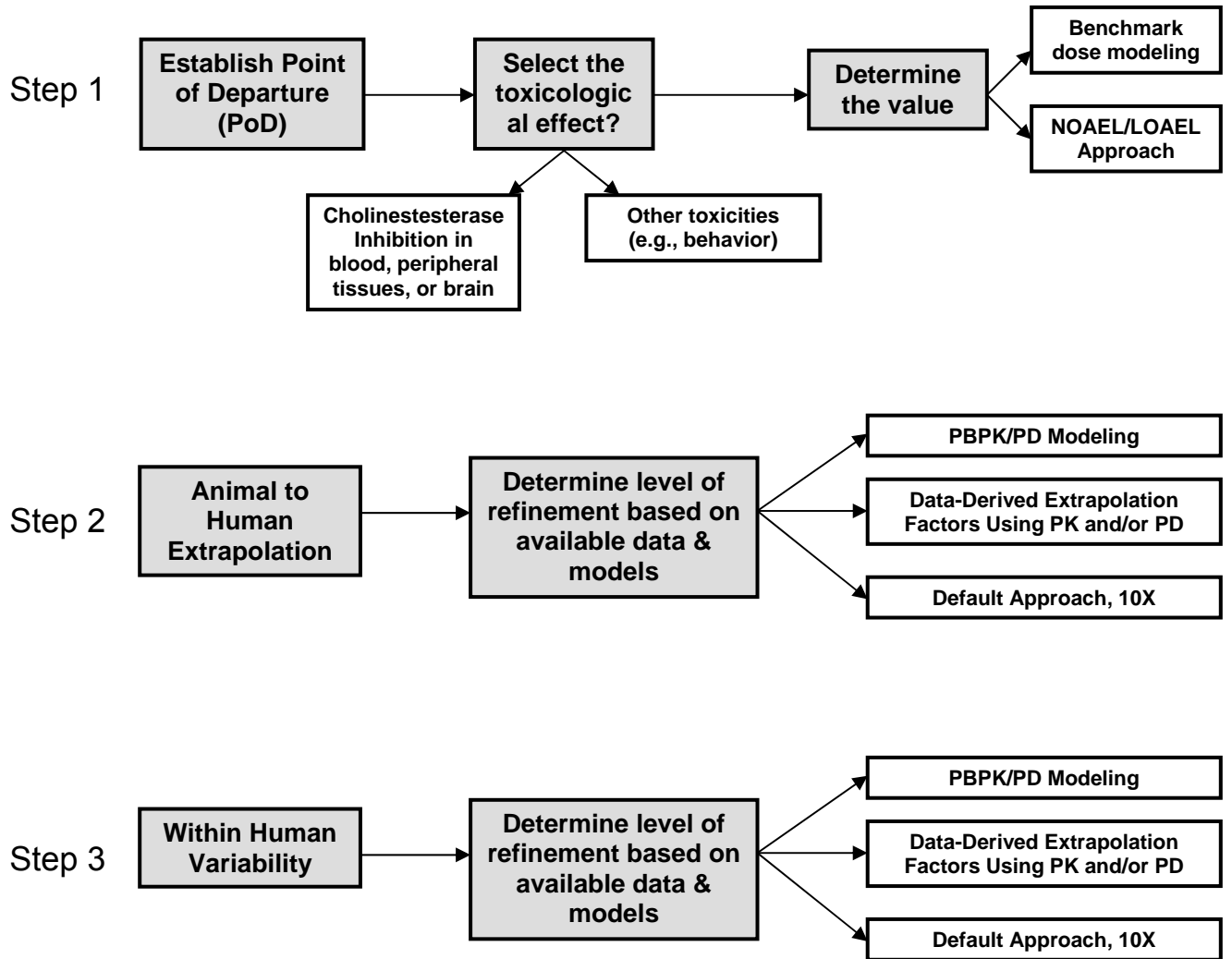
In recent years, U.S. and international efforts have made significant improvements in the scientific basis for human health risk assessments by increasing the use of mechanistic and kinetic data, and the Agency has emphasized the use of mode of action information in characterizing potential health effects of exposure to environmental agents (US EPA, 2005). International efforts including ILSI's (International Life Sciences Institute) and the International Programme on Chemical Safety's (IPCS) human relevance framework for evaluating the qualitative and quantitative relevance of a particular animal model of action in humans discuss the use of chemical specific and generic data when considering animal to human extrapolation (Seed et al, 2005; Meek et al 2003, Boobis et al, 2008). The IPCS guidance on developing Chemical Specific Adjustment Factors (CSAFs; WHO, 2005) describes the use of kinetic and mechanistic data to derive inter- and intra-species extrapolation factors based on data instead of reliance solely on default factors. Consistent with these efforts, this issue paper and the associated appendices emphasize toxicokinetics (TK) and toxicodynamic (TD) data to evaluate and apply the new chlorpyrifos research from animals and humans.

Figure 1 provides a schematic of the key steps involved in the update to the chlorpyrifos hazard/dose-response characterization. The first step involves selecting a point of departure (PoD). A PoD is a point on the dose-response curve which is at the low end of the observable data and is used as the starting point for extrapolation. A key component of hazard assessment is the selection and determination of the critical effect for predicting and estimating human health risk. The Agency's goal is to select a critical effect that will be protective for other toxicities. Depending on the compounds chemical and toxicity profile/characteristics, different critical effects may (or may not) be

¹ The Agency notes that the NRDC petition includes multiple science issues, many which are addressed in this issue paper. Some other issues, particularly results of the Agricultural Health Study and exposures related to volatilization of chlorpyrifos are not addressed here but are being addressed in other on-going efforts.

manifested for different durations of exposure, routes, or lifestages. Historically the Agency has selected PoDs for chlorpyrifos and other OPs based on inhibition of acetylcholinesterase (AChE). An important component of the current analysis is the comparison of doses causing inhibition of AChE at various durations, routes, and lifestages with doses resulting in other toxicities.

Figure 1. Schematic of decision points in the updated chlorpyrifos hazard identification



PoDs can be no-observed-effect-levels (NOAELs), lowest-observed-effect-levels (LOAELs), or derived from benchmark dose (BMD) modeling. The use of BMD modeling to derive PoDs is the preferred approach when sufficient data are available to reliably support modeling. The NOAEL/LOAEL approach historically used by EPA does not account for the variability in the experimental results, which are due to characteristics of the study design, such as dose selection, dose spacing, and sample size. This paper includes BMD modeling on selected AChE studies using a similar dose-response model (i.e., decreasing exponential) used in the OP and *N*-methyl carbamate cumulative risk assessments and supported by the FIFRA SAP on multiple occasions (FIFRA SAP 2001, 2002, 2005a, 2005b, 2008). BMD estimates are provided for pregnant rats and young post-natal (PN) rat pups (post-natal day one or PND1). The Agency plans to extend this analysis up to pups up to age PND 17. BMD modeling has not been attempted by the Agency thus far on data from other toxicities due to lack of dose-response data amenable to BMD modeling in the majority of such studies.

The next steps in the updated hazard/dose response characterization of chlorpyrifos involve consideration of animal to human extrapolation and within human variability. The preferred approach for extrapolating from the PoD to lower doses is to incorporate mode of action information and use sophisticated models like physiologically-based pharmacokinetic (PBPK)/ pharmacodynamic (PD) or biologically-based dose-response (BBDR) models. When such models are not available, uncertainty or extrapolation factors are used typically used to extrapolate from animal to humans (i.e., inter-species) and among the human population to account for sensitive individuals (i.e., intra-species). Historically, the Agency has used default 10-fold factors to account for inter- and intra-species extrapolation. More recently, with increased emphasis on the use of TK and TD data in risk assessment, the derivation of uncertain factors (UFs) derived from data, instead of default factors, has also increased. With the intent of improving the scientific basis for the chlorpyrifos risk assessment, in this issue paper the Agency has considered the availability of current models and mechanistic data for chlorpyrifos to use in animal to human and within human extrapolation. Overall, the available PBPK models, although well-developed and supported for non-pregnant adults, do not include calculations for pregnancy (e.g., no placental compartment) and for young children less than 5 years old and thus can not be used in a quantitative manner in extrapolation in this analysis.

In the absence of PBPK models for use in extrapolation, the Agency proposes to use chemical specific adjustment factors derived from data over the application of the 10X default factors. As such, the Agency has used the 2005 IPCS guidance on Chemical-Specific Adjustment Factors to evaluate available TD and TK data in animals and humans and to determine the extent to which such data support data-derived or chemical-specific extrapolation factors. In short, the Agency has concluded that with regard to TD characteristics, due to likelihood of different mechanisms of toxicity of chlorpyrifos and lack of identifiable and measurable key events for modes of action not related to AChE inhibition, the Agency proposes not to refine the TD component of the animal to human and within human variability factors. The Agency further proposes that the TK component of the animal to human factor can not be refined. This determination was based in large part of key differences in rat and human development with regard to the temporal maturation of detoxification enzymes and the related

difficulty in matching appropriate TK parameters for rats and humans. Due to the availability of extensive data on paraoxonase-1 (PON1) in many populations worldwide and in different lifestages, the Agency has performed a series of calculations to assess within human variability for TK with regard to PON1 activity.

This issue paper 1) summarizes data relevant to infants, children, and pregnant women from many areas of research, 2) provides an interpretation of these data in the context of human health risk assessment, and 3) offers options for updating the PoD(s) and extrapolation factors (intra- and interspecies UFs) for chlorpyrifos. In this document, proposed PoDs are UFs are discussed first followed by summaries of information on metabolism, AChE inhibition, effects on the developing brain in experimental animals, and epidemiology of children exposed pre- or post-natally. These summaries provide an integrative synthesis of available information and preliminary conclusions about specific aspects of the data that are most relevant to the selection of PODs and extrapolation factors.

Seven appendices to this issue paper contain an extensive review of the scientific literature and provide the background, scientific support, and analyses performed so far by the Agency:

- Appendices A-D describe metabolism and pharmacokinetics, acetylcholinesterase (AChE) inhibition data, other modes of action and toxicities besides AChE inhibition, and epidemiological studies in mothers and children.
- Appendix E provides an analysis of data for chlorpyrifos which may be used to inform data-derived inter- and/or intra-species extrapolation factors.
- Appendix F provides the results of benchmark dose modeling on selected studies with sufficient dose-response information.
- Appendix G provides draft data evaluation records (DER) for deliberate dosing studies with human subjects.

Appendices A-E primarily contain summary information and which represent varying levels of detail and discussion. The literature reviews contained in the appendices are not meant to be exhaustive review but instead are meant to describe key studies and findings.

This document is not a full and complete risk assessment/characterization. This paper does not address the FQPA 10X factor for infants and children. The Food Quality Protection Act (FQPA, 1996) requires the Agency to add a presumptive additional 10X safety factor for the protection of infants and children to take into account, among other things, the “completeness of data with respect to exposure and toxicity to infants and children (emphasis added).” EPA may assign a safety factor different than 10X if reliable data show that factor to be safe. In making this determination, EPA considers both toxicity and exposure issues. Since an exposure assessment is not included here, a complete analysis for the FQPA 10X is also not discussed here. It is important to note that the Agency has not developed any final conclusions regarding updates to the chlorpyrifos hazard assessment. At this time, the

Agency has progressed to a stage where feedback and peer review on the overall direction of the assessment is warranted.

2.0 Updated Points of Departure & Uncertainty Factors

2.1. Pathways of Exposure

In any risk assessment, before the PoDs and UFs are determined, it is important to first consider the relevant exposure scenarios or pathways (by route and duration), what population groups are exposed, and how they are exposed. This information guides the relevant toxicity data needed to evaluate the exposure scenarios of interest from different routes, duration of exposure, and lifestyles.

- *What uses remain for chlorpyrifos?*
The remaining uses of chlorpyrifos are primarily agricultural.
- *What routes of exposure are relevant for chlorpyrifos?*
The general population can be exposed to chlorpyrifos via the oral route in food and water (i.e., from run-off or leaching). Exposure (via the inhalation and/or dermal routes) may also occur through volatilization and spray drift for those people living in close proximity to fields treated with chlorpyrifos. Workers who apply or handle chlorpyrifos are exposed via the inhalation and dermal routes. To the extent possible, it is preferred to use route specific data when such data are available as TK properties (e.g., absorption) vary among the dermal, inhalation, and oral routes and route specific data helps account for these TK differences. Gestational and post-natal studies in pups are rarely available via the dermal and inhalation routes, particularly at low doses. As such, route-to-route extrapolation may be necessary for these routes.
- *What age groups or lifestyles need to be considered?*

For the general population, all age groups could be exposed in the diet. The following age groups are typically analyzed by OPP: infants (children <1); 1 to 2 years old; 3 through 5 years old; 6 through 12 years old; 13 through 19 years old; 20 through 49 year olds; 50 years of age and greater; and females of childbearing age (13-49 years old). These age groups were selected since they provide a broad representation of potential exposures. For this effort, the Agency has focused on children (<1-18 years old) and females of childbearing age as these groups represent potentially susceptible populations.

For workers, adults are evaluated. OPP does not develop separate exposure or risk estimates for female and male workers but instead assesses the more sensitive sex. In the case of chlorpyrifos, females are the more appropriate sex for consideration as they may be or could become pregnant when chlorpyrifos application or post-application activities occur.

- *What duration(s) of exposure are appropriate?*

For dietary risk assessments for single chemical risk assessments, OPP typically evaluates acute (24 hour) and chronic (1 year) exposures.

Worker risk is evaluated by short- (1-30 days), intermediate- (30 days-6 months), and long-term (>6 months to a year) exposure durations. The appropriate duration of exposure for worker risk is determined by the exposure pattern of a particular chemical and the relevant worker activities for mixing/loading/applying and/or post-application activities (e.g., thinning, picking). It is not unusual for workers to be exposed to the same pesticide for repeated days.

2.2. Summary of 2000 Human Health Risk Assessment

To provide context to the reader, the PoDs and UFs used in the 2000 risk assessment are shown below. Some of the scenarios evaluated in 2000 are not relevant due to the cancellation of many residential uses. For the 2000 human health risk assessment, EPA evaluated the available registrant submitted studies and scientific literature and identified NOAELs and LOAELs based on statistically significant plasma and RBC ChE inhibition for use in risk assessment. Blood ChE inhibition was used as the endpoint for all scenarios. Doses from adult animals were selected from oral, dermal, and inhalation studies for use in route specific risk assessments. Table 1 represents a summary of the toxicological endpoints for acute oral, chronic oral, dermal and inhalation scenarios from the 2000 risk assessment.

Table 1. Toxicological endpoints and uncertainty factors selected in the 2000 human health risk assessment for chlorpyrifos

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (single day)	NOAEL=0.5 UF = 100 FQPA = 10	1 mg/kg/day: 40% plasma cholinesterase inhibition at peak time of inhibition (6 hours post exposure) in adult males (RBC not measured) (Mendrala and Brzak 1998) 1.5 mg/kg/day: Significant 23.7% plasma and 29.7% RBC ChE inhibition at 4 hours post exposure in adult males	Blood Time Course Study (Mendrala and Brzak 1998, 44648102) with support from Zheng et al. (2000)
Chronic Dietary	NOAEL= 0.03 UF= 100 FQPA = 10	Significant Plasma and RBC cholinesterase inhibition at 0.2 to 0.33 mg/kg/day 0.22 mg/kg/day: Significant 33-67% ↓ plasma and 24-46% ↓ RBC ChE activity (90 day dog, Barker 1989); 0.3 mg/kg/day: 43% ↓ plasma and 41% ↓ RBC ChE activity relative to controls (2-week DNT Hoberman et al. 1998a,b) and 52% ↓ plasma and 39% ↓ RBC ChE activity relative to controls (2-week DNT companion study, Mattsson et al. 1998) 0.33 mg/kg/day: Significant 15-51% plasma ChE inhibition in both sexes, 19-31% RBC ChE inhibition at 104 weeks vs. controls (2-yr rat, Crown et al. 1990)	Weight of Evidence from 5 studies: <ul style="list-style-type: none"> • 2 year dog: McCollister et al. 1971/ Kociba et al. 1985, MRIDs 00064933/00146519; • 90 day dog: Barker 1989, MRID 42172801; • 2 yr rat: Crown et al., 1990, MRID 42172802; • 90 day rat: Crown et al. 1985, MRID 40436406; rat DNT: Hoberman et al. 1998a,b, MRID 44556901; rat DNT companion: Mattsson et al. 1998, MRID 44648101.
Short-Term Dermal (1-30 days)	Dermal NOAEL =5	Plasma and RBC cholinesterase inhibition of 45 and 16%, respectively at 10 mg/kg/day following 4 days.	21-day dermal rat and 4 day probe study (Calhoun and Johnson 1988, MRID #: 40972801)
Intermediate- and Long-Term Dermal (1 month to chronic)	Oral NOAEL =0.03	Plasma and RBC cholinesterase inhibition. 3% dermal absorption factor is required due to the use of an oral NOAEL (b).	Weight of Evidence from 5 studies (see chronic dietary above): 2 year dog; 90 day dog; 2 yr rat; 90 day rat; developmental neurotoxicity
Short-, and Intermediate-Term Inhalation (1 day to 6 months)	Inhalation NOAEL= 0.1	Lack of effects in 2 rat inhalation studies at the highest dose tested.	Two 90 day rat inhalation studies (Corley et al. 1986a,b MRID #: 40013901 & 40166501; Newton 1988, MRID #: 40908401 Makhteshim-Agan)

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Long-Term Inhalation (> 6 months)	Oral NOAEL= 0.03 100% absorption (relative to oral absorption)	Plasma and RBC cholinesterase inhibition	Weight of Evidence from 5 studies (see chronic dietary above): 2 year dog; 90 day dog; 2 yr rat; 90 day rat; developmental neurotoxicity

UF = Uncertainty Factor; a) Use absorbed dermal NOAEL of 0.15 mg/kg/day (5 mg/kg/day * 0.03 dermal absorption factor) for comparison with absorbed biomonitoring exposure.

2.3. Data Available for Consideration

The toxicity studies considered in the Agency's review are summarized in below in Section 3.0 and in Appendices B, C, D, E, and G. The scientific literature on chlorpyrifos is extensive and includes data from many sources, in animals and humans, and includes guideline studies with standard protocols and literature studies with atypical study designs. Sources of human information include deliberate dosing studies, epidemiology studies, and metabolism studies (*in vitro* and *in vivo*). Like many pesticide chemicals, there are a variety of studies evaluating different durations of exposure, different animal species, systemic toxicity, reproductive and developmental toxicity, neurotoxicity, and developmental neurotoxicity (DNT). A significant component of the current review includes numerous literature studies which have considered many different effects in multiple species. Many of the animal studies from the literature involve unusual study designs, measure unique biological parameters or outcomes, and use novel techniques. Many of the studies considered in this review use only a single dose for a particular age or lifestage and many others only use high doses at or near lethality. Studies that use only a single dose do not provide any information about the shape of the dose-response curve and those using only high doses may be less relevant when extrapolating human risk at low environmental exposures. Overall, the Agency has given more weight to studies and outcomes replicated by multiple laboratories and/or with multiple techniques and to studies which utilized relevant routes/durations of exposure and relatively low and/or multiple doses.

2.3.1. Human Information

2.3.1.1. Deliberate Dosing Studies in Human Subjects

Three deliberate dosing studies in adult (non-pregnant) humans are available which measure AChE activity and urinary levels of chlorpyrifos and/or its metabolites (See Appendix G). The Agency has determined that the deliberate dosing studies in adults are not appropriate for use in PoD or UF derivation in the current proposal. These studies provide valuable information on correlating oral or dermal exposure with levels of chlorpyrifos and/or TCP in blood and urine. In addition, these studies also provide information on time course of absorption, metabolism, and excretion. Kisicki et al (1999) also includes PON1 genotype information. Due to the availability of quality TK information, these studies have been used in the past by the Agency to aid in interpreting biomonitoring data. Specifically, results from Nolan et al (1982) have been used previously by the Agency in estimating (i.e., back-calculating) chlorpyrifos exposure based on urinary levels of TCP. Nolan et al (1982) has also been used to derive a dermal absorption factor in humans.

The blood ChE data from these studies have not been proposed for use in deriving PoDs or UFs. The reason why the blood ChE data from these studies are not proposed for use in deriving PoDs or UFs is based on several factors. First, there are experimental laboratory data that indicate that the developing nervous system may be susceptible to chlorpyrifos by mechanisms related to cholinergic and noncholinergic

mechanisms. Findings in epidemiology studies in children support the animal studies. These human studies do not include the potentially susceptible populations being evaluated in the current effort, namely pregnant woman and children and thus do not consider toxicity endpoints other than AChE inhibition (and related clinical signs). Nolan et al (1982) and Griffin et al (1999) only include a single dose group for a particular route. Griffin et al (1999) reports no changes in AChE inhibition and in Kisicki et al (1999) changes were only seen in one person. Studies with only a single dose group have been criticized in the past by the Human Studies Review Board (HSRB). Moreover, the HSRB has not supported the use of NOAEL studies in risk assessment since absence of an effect (LOAEL) raises questions about whether the investigators and the study design were able to detect an effect.

The Agency will solicit comment from the Panel on the Agency's preliminary decisions not to use the deliberate dosing studies with human subjects as a PoD or to reduce the inter-species UF but instead to use the TK data from these studies to help interpret biomonitoring and epidemiology studies. Depending on the comments by the Panel on these issues, the Agency may, if appropriate, take these studies to the Human Studies Review Board (HSRB) in the near future for the necessary scientific and ethical review.

2.3.1.2. Epidemiology Studies

One unique aspect of the chlorpyrifos literature is the availability of epidemiology studies in three cohorts of mothers and children. These studies are summarized in Section 3.D with more details provided in Appendix D. The epidemiology studies provide important information about potential human outcomes related to the potential effects of OPs on the developing brain. Moreover, they provide data which supports the human relevance of outcomes observed in the laboratory animal studies. They are not for use in directly deriving the PoDs or UFs for several reasons. The Agency is aware of an effort by Drs. Dale Hattis and Robin Whyatt to develop a PBPK model which includes a placental compartment for assessing tissue dosimetry to the fetus and which accounts for intra-species TK variability. The investigators then plan to use that model to estimate a human PoD from the blood biomarker reported in Whyatt et al (2003). This work has only just begun and will likely take several years. These epidemiology studies are, however, informative in considering factors which contribute to population variability and in evaluating the types of toxicities and their respective human relevance observed in animal studies.

Similar to many other epidemiology studies, these studies have not measured urinary or blood metabolites at or near the timing of pesticide applications. Each of the cohorts has been exposed to chlorpyrifos to some extent. There is some limited dose-response information that provides support for the contribution of chlorpyrifos to the birth and neurodevelopmental outcomes reported. The Columbia University team has correlated the timing of the voluntary cancellation of indoor uses of chlorpyrifos with maternal and cord blood levels of chlorpyrifos (Whyatt et al. 2003, 2004). They have further associated 'high' levels of chlorpyrifos with birth and neurodevelopmental outcomes. Since the voluntary cancellation of the indoor uses, maternal and umbilical

cord blood levels of chlorpyrifos in the Columbia University cohort have dropped substantially.

In addition to chlorpyrifos, each cohort has been exposed to multiple pesticides, including other OPs. This is particularly true for the study conducted at the University of California at Berkley where the cohort has been exposed to many OPs (Eskenazi et al. 2004, 2007; Bradman et al. 2003, 2005, 2007). The potential neurodevelopmental mechanisms, and the related dose responses, other than AChE inhibition are not well understood. As such, given the lack of a reliable biomarker of effect for these toxicities, determining the quantitative contribution of chlorpyrifos to the reported outcomes separate from the other OPs is challenging. With improved understanding, the Agency may, in the future, be able to better characterize the linkage between blood or urinary levels of chlorpyrifos and/or its metabolites with health outcomes. At this time, the Agency has used the reported levels of chlorpyrifos and its metabolites simply as markers of exposure without an attempt to link and estimate dose-effects relationships.

2.3.1.3. Studies on Metabolism and Toxicokinetics

A key component of this issue paper is the use of the 2005 IPCS guidance on Chemical Specific Adjustment Factors to evaluate available metabolic data for use in informing inter- and intra-species extrapolation factors. The Agency prefers to use extrapolation factors informed by data rather than applying default factors in order to improve the scientific support for a risk assessment. The metabolic profile of chlorpyrifos is well characterized in animals and humans, due, in part, to a large body of *in vivo* and *in vitro* metabolism studies from rats and humans (See Section 2.A and Appendix A). The Agency has evaluated the extent to which available data on carboxylesterase, butyryl cholinesterase (BuChE), PON1 (or A-esterase), and P450s are sufficiently robust to develop data-derived inter- or intra-species factors. This analysis is summarized in Section 2.E with more details provided in Appendix E.

In brief, there a variety of studies which inform human metabolism of chlorpyrifos. Human and rat metabolism studies in adults provide the major metabolites and the time course of absorption, metabolism and excretion. *In vitro* studies using human and rat tissues on multiple enzymes (carboxylesterase, BuChE, A-esterase or PON1, P450s) are available. Studies on metabolism and TK of chlorpyrifos provide characterization for interpreting gestational and post-natal toxicity studies as these studies provide data on tissue dosimetry and ontogeny of detoxification enzymes.

In general, the *in vitro* studies do not contain sufficient numbers of samples across different age groups and lifestages for quantifying within human variability. Inter-species extrapolation is challenging for gestational effects given differences in rat and human pregnancy with regard to birth and maturation of metabolic processes. There are uncertainties surrounding appropriate matching of rat and human information for purposes of extrapolation. However, there are extensive data on the population variability of PON1 which is being considered for use in addressing the TK component of the intraspecies factor.

2.3.2. Animal Studies

The Agency has focused its effort to derive PoDs on animal studies, particularly those in rats as rat because this is the species most often used in both literature and guideline studies. Sections 3.B and 3.C and Appendices B and C contain the Agency's review of available animal studies. The animal database on chlorpyrifos is large and includes evaluation a many different toxicities in multiple age groups and lifestages. The focus of the Agency's review has been on studies in pregnant animals and/or post-natal studies in juveniles as these groups represent potentially susceptible subpopulations.

The Agency's analysis contains two major components. The first component includes evaluation of AChE data from gestational and post-natal studies. This review includes both qualitative and quantitative considerations. First, a qualitative evaluation across many studies considered lifestage and age-related sensitivity and also a variety of issues such as duration of exposure and method/route of administration. The AChE review also includes a BMD analysis of selected studies (see below) which contain sufficient dose-response data and represent a variety of lifestages and age groups.

The second component of the review includes evaluation of toxicities not directly associated with AChE inhibition. Early in the review, the Agency had considered performing a MOA analysis using the MOA Framework (see website: http://www.who.int/ipcs/methods/harmonization/areas/non_cancer/en/index.html). However, it became clear in the initial review that sufficient data, particularly with respect to dose response and temporal concordance, are currently unavailable to perform a thorough MOA analysis. In the formal risk assessment, the Agency may consider doing a mode of action/human relevance framework analysis to identify key data deficiencies and research. In the mean time, the Agency is proposing to simply compare the dose levels used in these studies with those in AChE studies. This comparison informs the PoD determination as discussed below. In addition, the Agency has compared types of effects shown in animals with reported epidemiological outcomes. This comparison of animal studies and human outcomes provides support for the potential of chlorpyrifos at sufficiently high doses to result in effects on the developing brain.

2.4. Dose Response Assessment

PoDs can be NOAELs, LOAELs, or derived from BMD modeling (Figure 1). Numerous scientific peer review panels over the last decade have supported the Agency's application of the BMD approach as an improvement over the historically applied approach of using NOAELs or LOAELs and as a scientifically supportable method for deriving PoDs in human health risk assessment. The NOAEL/LOAEL approach does not account for the variability and uncertainty in the experimental results, which are due to characteristics of the study design, such as dose selection, dose spacing, and sample size (USEPA, 2000). With the BMD approach, all the dose response data are used to derive a PoD. For multiple AChE studies, there are sufficient dose response data to support a BMD analysis. This analysis is described below.

The Agency has not performed BMD analysis on studies evaluating the effect of chlorpyrifos on the developing brain as these do not provide dose response data amenable to BMD modeling analysis. Specifically, these studies, in general, may include only a single dose at a particular age, do not report graded responses (i.e., all or nothing effect), and/or show non-monotonic dose response curves (e.g., response goes up then down). For these studies, the Agency simply considered the doses used.

2.4.1. AChE Inhibition

As a preliminary analysis, the Agency has conducted BMD modeling on selected AChE studies described in Appendix B. These studies were selected based on the availability of at least two treatment groups. In addition, these studies were selected as they represented a variety of ages and durations of exposure. Selected studies include repeated gestational exposures to the dam and acute exposures to PND1 pups. The Agency is extending this analysis to include additional acute studies in pups up to age PND17 and to include repeated dosing studies in pups. More details of the BMD modeling reported here can be found in Appendix F.

The Agency has used a decreasing exponential dose-response model similar to that used for the OP and *N*-methyl carbamate cumulative risk assessments and previously reviewed and supported by the FIFRA SAP on several occasions (FIFRA SAP 2001, 2002, 2005a, 2005b, 2008). Consistent with risk assessment on other OP and NMCs compounds, the Agency has used a benchmark response level of 10% and has thus calculated BMD₁₀s and BMDL₁₀s. The BMD₁₀ is the estimated dose where AChE is inhibited by 10% compared to background. The BMDL₁₀ is the lower confidence bound on the BMD₁₀. Extensive analyses conducted as part of the OP cumulative risk assessment (USEPA, 2002) have demonstrated that 10% is a level that can be reliably measured in the majority of rat toxicity studies, and is generally at or near the limit of sensitivity for discerning a statistically significant decrease in AChE activity across the brain compartment and is a response level close to the background AChE level. The Agency uses the BMDL, not the BMD, for use as the PoD since the BMDL accounts for variability of the data (USEPA, 2000). The BMD₁₀ provides a point of comparison across studies. Table 2 provides the results of the preliminary BMD analysis.

Typically, studies submitted for pesticide registration and most studies from the public literature only measure brain and/or blood ChEs. It is rare for data from peripheral tissues to be available for consideration. Chlorpyrifos is unique in that multiple studies are available which provide such peripheral data. Table 2 does not include BMD results for plasma ChE measures. Consistent with OPP's ChE policy, plasma ChE data from animals are used for risk assessment when RBC AChE data are not reliable and/or when peripheral AChE measures are not available. This is not the case for chlorpyrifos; reliable RBC and peripheral data are both available.

As shown in Table 2, the preliminary BMD analysis has included brain, blood, and peripheral (heart) ChE inhibition data. As of August, 2008, the Agency had completed a preliminary analysis of the registrant submitted studies (Hoberman et al.

1998a, b, Mattsson et al. 1998, MRID44556901, 44648101, Maurissen et al. 2000, Mendrala and Brzak 1988), in addition to published literature studies by Betancourt and Carr (2004), Zheng et al (2000), Moser et al (2006), and Timchalk et al (2006).

Data from Zheng et al (2000), Moser et al (2006), and Timchalk et al (2006) were provided by the authors to OPP for analysis. Regarding the Timchalk et al (2006) data, the Agency has not reported BMD₁₀/BMDL₁₀s for the PND5 chlorpyrifos treated groups (1 and 10 mg/kg) because the values for some individual animals at this age group were inconsistent with all other data in the study (reported to have 3X more activity than other animals of same age). BMD estimates from Zheng et al (2000) are also not reported. A preliminary analysis was conducted on Zheng et al (2000). The BMD estimates are considered low confidence because the AChE data in this study at low doses at or near 10% inhibition are highly variable. Analysis of PND17 data from Moser et al (2006) and Timchalk et al (2006) will be submitted to the Panel in a supplemental report. In addition, BMD analysis of repeated dosing studies in post-natal pups will be provided to the Panel in a supplemental report.

The Agency has only reported BMD₁₀ values for acute dosing from Betancourt and Carr (2004). The published report for this study does not provide all the necessary information needed for a complete BMD analysis, particularly the lower 95% confidence limits (i.e., BMDL₁₀). Specifically, the actual sample size and standard deviation for all treatment groups were not reported in the publications (ranges were reported for sample sizes; standard error were reported for some groups but not all). The Agency has estimated the missing values based on the results from a variety of studies from Russell Carr's laboratory at Mississippi State University in the analysis.

For acute post-natal exposures in PND1 and 12, the brain BMD₁₀s are 0.12 and 0.64 mg/kg, respectively. The BMD₁₀ for RBC AChE in PND12 pups 0.25 mg/kg. There are no peripheral data available in pups amenable to BMD modeling. However, given the remarkable similarity in response between peripheral (heart) and RBC AChE data in dams, use of RBC AChE data from PND12 pups as a surrogate for peripheral data is reasonable. Among the BMD₁₀s estimated for brain AChE inhibition in repeated dosing adult studies, there is little variability across the studies. Specifically, BMD₁₀s from gestational studies in dams range between 0.5 and 1.2 mg/kg/day. For dams, BMDL₁₀s for RBC and heart AChE are approximately 10-fold lower than values for brain AChE inhibition. Like the brain AChE results, little variability is also seen among the blood and heart AChE data in dams where BMD₁₀s range from 0.06 to 0.16 mg/kg/day.

Table 2. Summary of Benchmark Dose Analyses for Acute and Repeated AChE Studies in Rat

Reference	Age	Brain (mg/kg/day)		RBC (mg/kg/day)		Heart (mg/kg/day)	
		BMD ₁₀	BMDL ₁₀	BMD ₁₀	BMDL ₁₀	BMD ₁₀	BMDL ₁₀
Acute/Single Dose Studies							
Betancourt and Carr (2004)	PND 1	0.12	NR	NA			
Timchalk et al. (2006)	PND 12	0.64	0.54	0.25	0.08	NA	
Repeat Dose Studies							
2006 Cumulative RA	Repeated >21 days, Adult Female (non-pregnant)	1.48	1.26	NA			
Dow (Hoberman et al. 1998a,b, MRID44556901); Maurissen, 2000	Dam, GD6-20	0.65	0.54	0.06	0.03	NA	
Dow (Mattsson et al. 1998 44648101); Mattsson, 2000	Dam, GD6-20	Hindbrain 1.10 Forebrain 1.17	Hindbrain 0.81 Forebrain 0.98	0.14	0.08	0.16	0.12
Dow (Mattsson et al. 1998 44648101); Mattsson, 2000	Dam, LD 1	Hindbrain 0.96 Forebrain 1.11	Hindbrain 0.55 Forebrain 0.77	0.079	0.0498	0.109	0.056

NA= Not applicable;

2.4.2. Effects on the Developing Nervous System

While there are a number of studies demonstrating long-term effects of peri-natal chlorpyrifos exposure, most of those studies were conducted with only one or two dose levels, and often both doses showed similar effects. Thus, the data are not amenable to benchmark dose modeling. As discussed in Section 3.3 and Appendix C, when comparing across studies, these studies provide a basis for concern that a dose of 1 mg/kg/day, in rats and mice, administered for as few as four consecutive days will produce a variety of neurobehavioral outcomes in the offspring. These effects at 1 mg/kg are summarized in Table 3.

Across all studies, the lowest tested dose tested was 0.3 mg/kg/day. This dose was used as the lowest dose in the DNT study, which involved repeated oral exposures to the dam through gestation and until postnatal day 10. No behavioral effects were observed at any time in the offspring exposed to this dose level, although the Agency notes that brain morphometric changes (seen at 1 mg/kg/day) are not available from this dose group. This same dose was used by Jett et al (2001) dosed every fourth day on PND 7, 11 and 15 in one group of rats and on PND 22 and 26 in a second group. Jett et al (2001) used subcutaneous injection in peanut oil. Significant effects on learning in a Morris water maze was observed in the 0.3 mg/kg/day dose group, but only when they were receiving the treatment while testing was being conducted (PND 24-28). Thus, these results may have been influenced by the concurrent dosing and reflect acute toxicity of chlorpyrifos. For these reasons, 0.3 mg/kg/day cannot be confidently used as either a NOAEL or LOAEL.

Table 3. Summary of tests in Adults (at least 5 weeks of age) Following Gestational and/or Early Postnatal Dosing of 1 mg/kg/day Chlorpyrifos (excluding effects of higher doses). All data are in rats except where indicated.

Behavioral Domain	Device/Task	Outcomes at 1 mg/kg/day
Locomotor activity	Figure-8 maze	Rate of habituation increased or decreased, depending on exposure window and gender
	Open-field (mice)	Increased
	T maze	Decreased early in training
	Elevated plus maze	Increased with PND 1-4 dosing
	Radial arm maze	Not altered
Learning & Memory	T maze	Not altered
	Radial arm maze	Increased errors early in training; decreased errors in females exposed PND 1-4; not altered with PND 11-14 dosing
	T maze	Not altered
	Passive avoidance (mice)	Not altered
Neuromotor response	Acoustic startle	Not altered
Social Interactions (mice)	Conspecific behaviors	Not altered
	Recognition	Not altered
	Agonistic behaviors (male)	Increased
	Induced maternal behaviors (female)	Increased
Depression	Elevated plus maze	Increased time in open arms (both species but gender differences)
	Chocolate milk preference	Decreased
Neurotransmitter system involvement	Cholinergic	Muscarinic subsensitivity; no change in nicotinic
	Serotonergic	Abnormal sensitivity

2.5. Proposed Points of Departure (PoD)

As discussed above, chlorpyrifos exposure is expected to occur through the oral, dermal, and inhalation routes. PoDs are needed for each route.

2.5.1. Oral Route

The Agency has proposed three options for the oral PoDs. The Agency will solicit comment on these options as well as alternative approaches.

Option 1: The first option proposes to use the PoDs which were based on rat RBC and plasma ChE inhibition in the 2000 risk assessment for acute oral, dermal and inhalation exposures, and rat and dog blood AChE for chronic oral exposures (Table 2). The 2000 risk assessment included a weight of the evidence discussion primarily on adult rat and dog AChE guideline studies and adult data from Zheng et al (2000). This option would involve application of the NOAELS for blood AChE inhibition from route specific studies (oral, dermal, inhalation) in rats or dogs to all populations. The acute oral PoD would be 0.5 mg/kg/day and the repeated oral PoD would be 0.03 mg/kg/day. The dermal and inhalation NOAELs would be 5 and 0.1 mg/kg/day, respectively.

Option 2: As mentioned above, some AChE studies provide sufficient dose response data amenable for BMD modeling, the preferred approach to deriving PoDs. AChE studies in repeated gestational studies in dams (heart) and acute post-natal pups (brain and RBC) provide BMD₁₀ and BMDL₁₀ values in the same range---0.06-0.12 mg/kg/day. In Option 2, the Agency proposes to use a PoD of 0.1 mg/kg/day for oral exposure of all age groups and all durations (i.e., acute and chronic).

This value of 0.1 mg/kg/day is not derived from a single value. Instead, the proposed value of 0.1 mg/kg/day is derived from a weight of the evidence using BMD_{10s} and BMDL_{10s} from brain and RBC AChE in young pups (PND1 and 12) in acute studies and peripheral (heart) AChE in repeated gestational studies with dams. As such, multiple lifestages are considered in the proposed PoD: pregnant dams, PND1 pups, and PND12 pups. With regards to the developing brain, PND 12 pups are similar to newborns. It can be argued that direct dosing of PND1 pups does not directly match human exposures since PND 1 pups are closer to third trimester human fetuses. However, the PND 1 data add support that very young animals may be more sensitive than adults following acute exposures. The proposed PoD of 0.1 mg/kg/day is also supported by the PND 12 blood data. Furthermore, the proposed value is 3-10 fold lower than causing effects on the developing brain reported in other laboratory animal studies and thus is expected to be protective for those effects. The lowest dose used in any study evaluating the potential for chlorpyrifos to affect learning/memory is 0.3 mg/kg from Jett et al (2001). The proposed PoD of 0.1 mg/kg/day is 3-fold lower (i.e., more health protective endpoints) than this dose of 0.3 mg/kg/day. The Agency further notes that these BMDL₁₀ values are approximately 10-fold lower (more health protective) than the lowest dose (1 mg/kg/day) used in many gestational and post-natal studies evaluated toxicities other than AChE inhibition (See Appendix C).

Thus, the Agency believes that the proposed PoD of 0.1 mg/kg/day provides a scientifically supportable value because it was derived 1) BMD modeling which is preferred over the use of NOAELs/LOAELs, 2) incorporates information from multiple susceptible lifestages, 3) and is protective of toxicities other than AChE inhibition, including behavioral effects remaining in adulthood.

Option 3: The third option is a blend of Options 1 and 2 and separates the PoD for acute and repeated exposures. The Agency proposes to use the same PoD of 0.1 mg/kg/day discussed above for all populations but only for acute oral exposure and for short-term dermal exposure to workers (1-30 days) as discussed in Option 1. For chronic dietary exposure, a PoD of 0.03 mg/kg/day for all populations is proposed. This is the same PoD used in the 2000 risk assessment. This value of 0.03 mg/kg/day is based on the NOAELs and LOAELs for plasma and RBC ChE inhibition in five studies (2-year study in dog, 90-day study in dog, 90-day study in rat, 2-year feeding in rat, the DNT study). This value of 0.03 mg/kg/day is supported by the BMDL₁₀ of 0.03 mg/kg/day in pregnant dams for RBC AChE inhibition (Table 2).

2.5.2. Dermal & Inhalation Routes

Route specific data are preferred because such data accounts for potential differences in absorption, distribution, or metabolism. In the case of chlorpyrifos, dermal and inhalation studies are available which identify NOAELs for these routes in adult rats. With respect to inhalation exposure, there are two nose only studies with vapor chlorpyrifos which provides a NOAEL of 287 ug/m³ or 20 ppb (0.1 mg/kg/day). Similarly, there are two dermal studies which together provide a dermal NOAEL in adult rats of 5 mg/kg/day. These studies do not include pregnant dams, fetuses or post-natal pups and therefore do not consider potentially susceptible populations. In the absence of data in these groups, the Agency will continue to use route specific studies, as appropriate. An alternative for dermal exposure is to use an oral PoD derived from susceptible populations (as discussed above) with a dermal absorption factor. Specifically, the Agency could use a dermal absorption of 3% from human subjects (Nolan et al, 1982). The Agency will solicit comment from the Panel what toxicity and/or TK studies (if any) in pregnant dams, fetuses, and/or post-natal pups could be conducted to better inform the dermal and inhalation risk assessments.

2.6. Extrapolation/Uncertainty Factors

In previous risk assessments, the Agency has applied the default 10X factors for both inter- and intra-species extrapolation in addition to the FQPA 10X. In 2005, the WHO published its guidance for deriving chemical specific adjustment factors (CSAFs; WHO, 2005). The guidance is based in large part on analyses by Renwick (1993) and Renwick and Lazarus (1998) and describes the use of TK and TD data in lieu of the default 10X safety factors for human sensitivity and experimental animal-to-human extrapolation. EPA has an on-going effort to develop similar guidance and has used these concepts in some risk assessments, including several pesticides.

As discussed in detail in Appendix E, the Agency has applied the 2005 IPCS Chemical Specific Adjustment Factors Guidance in the chlorpyrifos analysis. Understanding MOA is an important component of deriving data derived extrapolation factors (DDEFs) in that MOA provides the foundation for understanding which TK and TD factors are critical for extrapolation. Although, inhibition of AChE is a well established neurotoxic mode of action for OPs (including chlorpyrifos), chlorpyrifos may have multiple modes of action resulting in its effects on the developing nervous system. When deriving data-derived factors, it is necessary to consider each mode of toxicity, critical effect, target organ, and lifestage because the magnitude of extrapolation factors may differ among different toxicities and life stages.

The MOA involving inhibition of AChE leading to clinical signs of neurotoxicity and changes in behavior has been well-documented for many OPs. If AChE inhibition was the only mode of action affecting toxicity of chlorpyrifos, it may be possible to derive extrapolation factors for the TD component of animal to human (UF_{AD}) and possibly within human variability (UF_{HD}) because there are data which describe the molecular structure and *in vitro* effects of the AChE in different species and data which evaluate the *in vitro* effects of juvenile and adult AChE inhibition. However, other potential modes/mechanisms that may affect the developing brain are less understood and none are sufficiently robust to establish key events with dose-response and temporal concordance. Given the remaining uncertainty regarding the modes(s) of action affecting the developing brain---and specifically differences in animals and humans and within human variability---the Agency has elected to not develop a DDEF for UF_{AD} or UF_{HD} . As such, the Agency is proposing to apply the default 3X for inter- and intra-species TD extrapolation (i.e., UF_{AD} and UF_{HD}).

As discussed in detail in Appendix E, the Agency evaluated the extent to which data on carboxylesterases, P450s, and paraoxonase (PON1, or A-esterase) support development of DDEFs of inter- and intra- TK extrapolation (i.e., UF_{AK} and UF_{HK}). Based on differences in rat and human pregnancy with regard to birth and maturation of metabolic processes, there are uncertainties surrounding appropriate metabolic parameters for animal to human extrapolation of juveniles. This uncertainty in combination with limited data precludes the development of a DDEF for inter-species TK extrapolation (i.e., UF_{AK}). Thus, the Agency proposes to apply the default 3X for UF_{AK} . Data on carboxylesterases are not sufficiently robust for intra-species TK extrapolation (i.e., UF_{HK}). Data on P450s are complicated by multiple enzymes each with its own maturation profile. Others have evaluated the P450 literature for use in derivation of child specific UFs with poor success (Ginsberg et al, 2004a). There are, however, extensive data on PON1 which allow for development of a factor for intra-species TK extrapolation (i.e., UF_{HK}). The Agency has reviewed data on PON1 activity and enzymes levels among different populations and age groups. These data show that age-related maturation of PON1 is a larger source of variability compared with different genetic polymorphisms among adults, particularly for the PON-192 Q/R polymorphism.

The Agency is proposing two options for the intra-species TK extrapolation factor (UF_{HK}): one option involves using a UF_{HK} derived from PON1 data and a second option involves using a default factor. Specifically, the Agency is considering the use a 12X

factor based on chlorpyrifos oxonase (CPOase) data in mothers and newborns (Holland et al, 2006) as the basis of the UF_{HK} . This factor was derived in a manner consistent with IPSC (2005); the ratio of the 50th percentile in mothers and 5th percentile in newborns was calculated.

There are uncertainties associated with use of PON1 data as the source of information for UF_{HK} . The Agency has provided an extensive discussion of this in Section 3.0 and in Appendix E. The Agency will solicit comment from the SAP on several science issues related to interpreting and using PON1 data.

Table 4. Potential composite factors for chlorpyrifos

Factor	Toxicokinetics	Toxicodynamics	Combined
Inter-species	3X	3X	10X
Intra-species	3X or 12X	3X	10X or 36X
Composite Factor			100X or 360X

2.7. Issues for the FIFRA SAP

The Agency has not developed any final conclusions regarding PoDs or UFs. At this time, the Agency has progressed to a stage in the review where feedback and peer review on the overall direction of the assessment is warranted. The Agency is soliciting comment from the panel on the proposed PoDs and UFs (data and approach) and also on aspects of the literature review which provides the foundation of the proposals.

The following sections summarize key information on the metabolic profile of chlorpyrifos, AChE data, other toxicities besides AChE inhibition, and epidemiology studies in children. More detailed descriptions of these data can be found in Appendices A-E

3.0. Summary of Key Data Used in PoD & UF Determination

3.1. Metabolism and Toxicokinetics

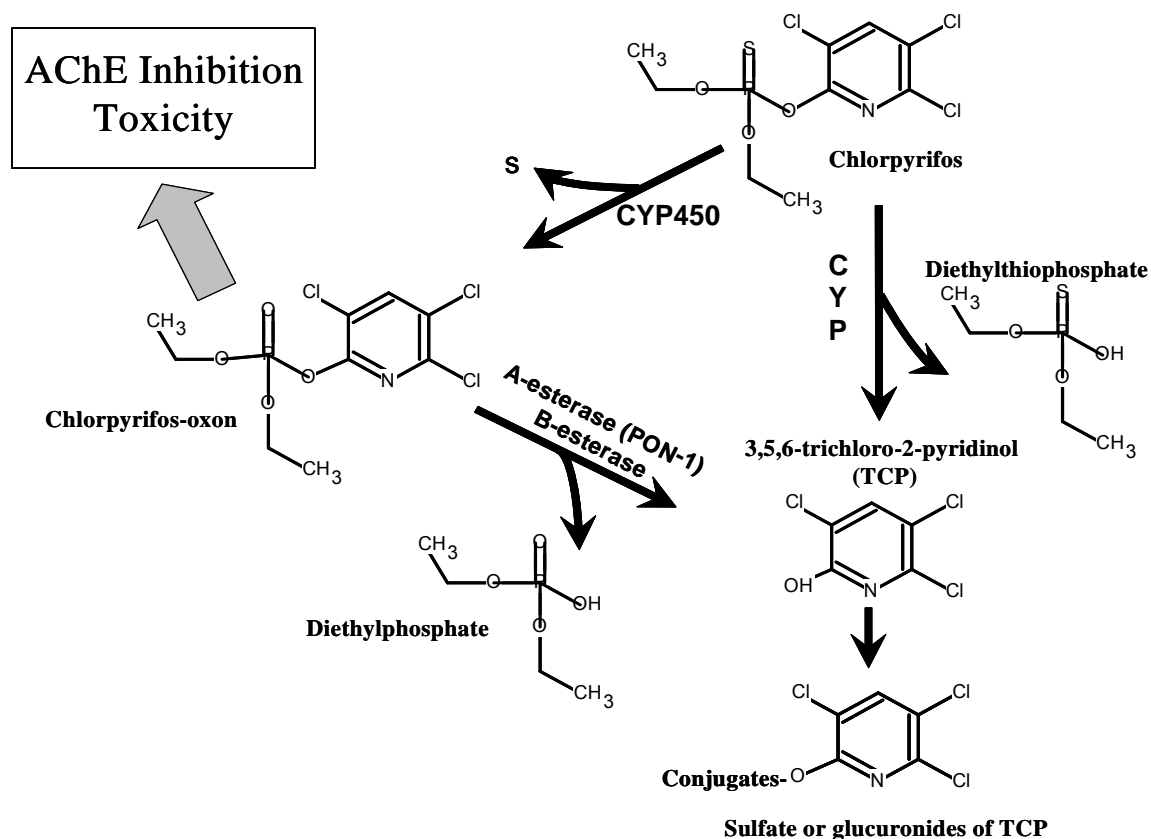
3.1.1. Metabolic Profile

The metabolism and TK of chlorpyrifos have been extensively studied in animals and humans as well as *in vitro* systems. Many of these studies are summarized in Appendix A. Overall, rats and humans show similar patterns of metabolism for chlorpyrifos in adults. Although less information is available to compare rats to humans with regard to pregnancy and post-natal maturation, as described below, the patterns of metabolism appear to be generally similar in rats and animals.

Chlorpyrifos undergoes metabolic transformations mainly by the liver enzymes residing in the microsomes. Although, chlorpyrifos is lipophilic, its extensive metabolism into water soluble metabolites does not lead to any accumulation of the parent material or its metabolites in the body tissues. The initial metabolic attack on the chlorpyrifos is its desulfuration, resulting in its bioactivation to the more toxic and potent AChE inhibitor, the oxon form (Figure 2). However, the oxon is unstable and is rapidly deactivated through hydrolytic cleavage by a process called dearylation releasing the 3,5,6-trichloro-2-pyridinol (TCP). Simultaneously along the desulfuration process, dearylation will be acting on both the parent chlorpyrifos as well as on the oxon metabolite leading to the release of TCP. TCP is further conjugated to form glycine or glucuronide conjugates and eliminated into the urine. TCP is the major excreted metabolite and used as the biomarker in PK, biomonitoring, and epidemiology studies.

There are several enzymes that play a role in the metabolism and toxicity of chlorpyrifos (Figure 2). In addition to inhibition of AChE, the oxon also binds stoichiometrically to butyrylcholinesterase (BuChE; abundant in blood and other tissues). In this regard BuChE is viewed as a scavenger of the oxon formed. The cytochrome P450 family of microsomal enzymes (CYPs) is responsible for its metabolic activation and deactivation. Another group of important enzymes in the detoxification of chlorpyrifos is the A-esterases; one such A-esterase is paraoxonase (i.e., PON1). These are calcium activated enzymes and are distributed in various tissues including the liver, brain and blood. These act on the oxon by hydrolyzing it before reaching its target AChE enzyme. The oxon also binds irreversibly to carboxylesterases. Carboxylesterases are distributed among different tissues (liver, blood, brain) with highest abundance in the liver. The glutathione dependent enzymes play an important role in the secondary metabolism of chlorpyrifos producing water soluble metabolites that are readily excreted into the urine.

Figure 2. Major metabolic pathways of chlorpyrifos metabolism (Reproduced from Timchalk et al, 2005)



3.1.1.1. Ontogeny of Metabolic Processes in the Young

Differential inhibition of the AChE enzyme itself does not appear to account for the observed age-related sensitivity found in young animals as suggested by *in vitro* studies (Benke and Murphy, 1975; Chanda *et al.*, 1995; Mortensen *et al.*, 1996; Atterberry *et al.*, 1997). Rat fetuses and juveniles and newborn humans, however, have lower capacity to detoxify than adults. This decreased capacity to detoxify has been associated with increased sensitivity in rats. Specifically, in rats, A-esterase activity is virtually nonexistent in the fetus (Lassiter *et al.*, 1998) and increases from birth to reach adult levels around PND21 (Mortensen *et al.*, 1996; Li *et al.*, 1997). Mortenson *et al.* (1996) showed that in the plasma level of CPOase² was 1/11 that of adult animals. The animal data regarding the role of carboxylesterase in mediating OP toxicity are also quite extensive (e.g., Clement, 1984; Fonnum *et al.*, 1985; Maxwell, 1992 a, b). Fetal rats possess very little carboxylesterase activity (Lassiter *et al.*, 1998) with increasing

² CPOase is A-esterase (PON1) activity specific to chlorpyrifos oxon

activity as the postnatal rat matures, reaching adult values after puberty (50 days-of-age; Morgan et al., 1994; Moser et al., 1998; Karanth and Pope, 2000). There are, however, very little data in human tissues which could evaluate age-related maturation of carboxylesterase expression. The available data come from Pope et al (2005) and Ecobichan and Stephens (1973). Ecobichan and Stephens (1973) showed a steady increase in AChE and ChE levels of infants beginning at birth up to adult levels. Pope et al (2005) evaluated maturational expression of liver carboxylesterases in human liver tissues from infants (2–24 months) and adults (20–36 years). The authors report relatively small (and not statistically significant) differences in activities between children ages 2–24 months and adults (20–36 years). The Agency notes, however, that youngest age evaluated in the study was 2 months old and this individual had the lowest level of carboxylesterase.

The temporal pattern of A-esterase activity (and carboxylesterases) correlates reasonably well with studies on OP sensitivity. Several studies have shown an increased sensitivity of newborn rats to OP compounds which are detoxified via the A-esterase and/or carboxylesterase pathways (Gagne and Brodeur, 1972; Benke and Murphy, 1975; Pope et al., 1991; Chambers and Carr, 1993; Padilla et al., 2000; 2002; Karanth and Pope, 2000).

There are only studies in the literature that have assessed A-esterase activity in children. Based on these studies, it appears that serum A-esterase levels are very low in human infants compared to adults (Augustinsson and Barr, 1962; Mueller et al., 1983; Ecobichon and Stephens, 1973; Holland et al, 2006; Chen et al, 2003). After birth, there is a steady increase of this activity (Augustinsson and Barr, 1963). In a related study of the age-dependence of total serum arylesterase (ARase) activity (of which a large component is A-esterase activity), adult levels were achieved by two years-of-age (Burlina et al., 1977). The Agency is aware of yet unpublished data of PON1 levels (A-esterase) in children up to age 5 from Drs. Nina Holland and Brenda Eskinazi with a much larger sample size (>200) than previous studies. These data were presented at the American Society of Human Genetics meeting (Huen et al, 2007) and suggest that paraoxonase activity may be lower than adult levels up to 47 months. After completion of the data analysis and ultimately publication, these data will substantially improve the overall understanding of the human ontogeny of PON1. Recent studies by Holland et al (2006) and Chen et al (2003) have provided ARase and/or CPOase activities in newborns and their mothers. These studies have been analyzed by the Agency as part of consideration of data derived intraspecies extrapolation factor for chlorpyrifos (Holland et al, 2006; Chen et al, 2003; Appendix E).

Maturation of the cytochrome P450s to detoxify or activate chlorpyrifos to the oxon may also play a role in age-related differences in the young and adults. The most important P450s for chlorpyrifos metabolism in humans are 1A2, 2B6, 2C19, and 3A4 (Buratti et al, 2002; Tang et al, 2001). It is important to note that CYP3A4 is deficient in neonates; the fetal form, CYP3A7, is active in utero and immediately after birth (LaCroix et al, 1997). In addition, CYP1A2 is barely detectable at birth and CYP 2C19 and 3A4 are 3 to 10-fold lower in newborns than other children and adults (Sonnier et al, 1998; Viera et al, 1996). Less is known about the development of CYP2B6 as this one is not as extensively studied as other CYPs.

3.1.1.2. Metabolic Changes During Pregnancy

Metabolic activities can be altered during pregnancy (Anderson 2006; Anger and Piquette-Miller 2008, Bologna *et al.* 1991; Carpintero *et al.* 1996; Czekaj *et al.* 2000 and 2005; Dickman *et al.* 2008; Ejiri *et al.* 2005; Ferre *et al.* 2006; Hines 2007; Homma *et al.* 2000; Howard and Sugden 1993; Tsutsumi *et al.* 2001)³. For example, Chanda *et al.* (2002) showed that pregnant female rats had lower plasma, brain, and liver carboxylesterase activity compared to non pregnant females. Regarding A-esterase (PON1) activity, Ferre *et al.* (2006) showed that the paraoxonase (POase) in serum decreased from a nonpregnant background of 146 U/L to 111 U/L in late gestation, indicating 76% of normal activity in late gestation pregnant women. Carpintero *et al.* (1996), however, found that phenyl acetate metabolism increased from 23.6 to 33.5 μ kat/g in the third trimester. Data in mice support the findings of Ferre *et al.* (2006) findings in humans suggesting a reduction in A-esterase activity during pregnancy. In mice, Weitman *et al.* (1983) found that PON1 activity towards parathion was 50 nmol/min/ml in non-pregnant females, but it decreased as low as 14 nmol/min/ml during gestation (Weitman *et al.*, 1983).

With regard to plasma ChE levels, Howard *et al.* (1978) have shown that in six healthy pregnant women levels of plasma ChE dropped by approximately 30% during the first trimester but returned to close to pre-pregnancy levels in the third trimester. Similarly, Venkataraman *et al.* (1990), Whittaker, *et al.* (1988), and De Peyster *et al.* (1994) both reported decreases in plasma ChE in pregnant women. Evans *et al.* (1988) showed that cholinesterase levels in 39 of 44 pregnant women dropped after conception; in 20 of those women, the decline in cholinesterase activity continued throughout pregnancy.

As mentioned above, the most important P450s for chlorpyrifos metabolism in humans are 1A2, 2B6, 2C19, and 3A4. Bologna *et al.* (1991) found that production of a marker substrate for 1A2 activity dropped to 41% of non-pregnant levels by the third trimester in epileptic women. While 1A2 and 2B activities decrease in pregnancy, 2C and 3A activities increase. In pregnant AIDS patients, endogenous cortisol metabolism (marker for 3A4 activity) increased by more than 2-fold (Homma *et al.*, 2000).

There is a consistent pattern for several key detoxification enzymes that metabolic activity may decrease during pregnancy. The reductions are not large in magnitude and the importance of these decreases is unknown at environmental exposures. However, these studies suggest the potential for a reduced capacity to detoxify during pregnancy. Toxicity studies in rats add further support that reduced ability to detoxify chlorpyrifos and/or the oxon effects sensitivity during pregnancy. Female rats, particularly pregnant rats, appear to be more sensitive than adult male rats to cholinesterase inhibition (Moser *et al.* 1998, Hoberman 1998a,b, Mattsson *et al.*

³ The Agency notes that metabolic changes during human pregnancy are also described in a public comment to the SAP docket by Drs. Torika Poet and Charles Timchalk of Battelle Laboratory.

1998). Moser and Padilla (1998) found that inhibition of ChE in brain tissues had a sooner onset, a later peak effect, and a slower recovery in adult (approximately PND 70) females administered a single oral gavage dose of 80 mg/kg, compared to males.

3.1.2. Data on Tissue Dosimetry

An important issue when evaluating gestational exposure is the extent to which the compound crosses the placenta and is thus available to affect fetal tissues. Cross placental tissue dosimetry data are rarely available in humans for pesticide chemicals. Umbilical cord data from one research group (Columbia University) are available for chlorpyrifos. Specifically, Whyatt et al (2003) have shown that levels of chlorpyrifos in maternal blood are similar to the levels measured in umbilical cord blood (Whyatt et al, 2003; Section 2.2). In gestational studies with rats, similar or higher levels of TCP in fetal brain and blood compared to dams suggests that chlorpyrifos and/or its metabolites reach the target tissue (brain) in the fetus (Hunter et al, 1999; Mattsson et al, 1998, 2000). Hunter et al, (1999) showed that the concentration of TCP in the fetal brain was 2-3- fold higher than the TCP concentration in the maternal brain in time course and dose-response studies. In a study by Mattsson et al (1998, 2000), concentrations of chlorpyrifos, the oxon, and TCP were measured in the blood of maternal and fetal tissues and in milk. Mattsson et al (1998, 2000) found that TCP levels in maternal and fetal blood were similar and chlorpyrifos levels were approximately 2-fold higher in maternal blood than fetal blood. The oxon is highly unstable and was only detected at high doses in the fetus (and not dams). In another gavage gestational exposure study, Akhtar et al (2006) exposed rats to chlorpyrifos from GD 0-20 in fetal and maternal tissues on GD21. It is difficult to make conclusions regarding dose relationships in this study due to a small range of dose. The investigators saw some inconsistent trends. Total residues of chlorpyrifos were higher in the fetuses than in the dams and brain concentrations of chlorpyrifos were greater or similar to dam levels. When considered together, these studies (Whyatt et al, 2003; Hunter et al, 1999; Mattsson et al, 1998, 2000; Akhtar et al, 2006) support the conclusion that chlorpyrifos and/or its metabolites are likely available to the fetus at similar (and possibly higher) levels compared with maternal tissues.

With regard to post-natal exposure to chlorpyrifos through breast milk, there is some limited data that show that chlorpyrifos can be found in breast milk. Mattsson et al (1998, 2000) provided data in rat milk which suggest that chlorpyrifos can reach milk at lower doses (0.3 mg/kg/day). There are very little human breast milk data in the literature except for persistence organic pollutants and none for chlorpyrifos from current U.S. exposure levels

3.1.3. Conclusions

TK studies from humans and rats support a preliminary conclusion that chlorpyrifos and/or its metabolites may be available to the fetus, likely at levels similar to maternal tissues. The Agency further concludes that TK differences in young and adults play an important role in the age-dependant sensitivity with chlorpyrifos (described below in Sections 3.B-D). Additional information in pregnant animals and

humans suggest that metabolic capacity to detoxify may be reduced during pregnancy, although the relevance of these changes is not known at low environmental levels.

3.2. Inhibition of Acetylcholinesterase (AChE)

Chlorpyrifos, like other OPs, binds to and phosphorylates the enzyme, AChE, in both the central (brain) and peripheral nervous systems leading to accumulation of acetylcholine and, ultimately, to clinical signs of toxicity. This mode of action, in which AChE inhibition leads to neurotoxicity, has been well described (Miles et al., 1998). In 2000, the Agency concluded for chlorpyrifos that inhibition of ChE was the most sensitive effect in all of the animal species evaluated and in humans, regardless of exposure duration. For the current analysis, the Agency has reviewed the studies submitted for registration as well as searched the public literature for studies in which pregnant animals and/or juvenile animals were exposed to chlorpyrifos. ChE inhibition is most commonly reported for the blood (plasma and RBC) and brain (whole or subsections), although a few studies have evaluated inhibition in peripheral tissues such as the heart, diaphragm, or lung. The following text provides a summary of key studies; more details, including extensive tables, can be found in Appendix B.

The Agency has examined time course information. In the available studies, the time to peak inhibition following exposure to chlorpyrifos was independent of age and method of exposure and varied from 2 to 24 hours across different studies but was typically between 3 and 6.5 hours. Recovery in young rats occurs faster than in adult animals (Chakraborti *et al.*, 1993; Moser and Padilla, 1998; Pope et al., 1991; Pope and Liu, 1997). In the fetus, comparison of Lassiter et al. (1998a) and Ashry et al. (2002) indicates that the time of peak brain ChE inhibition is the same (4-5 hours) following repeated (7 mg/kg/day) or acute oral exposure (50 mg/kg/day) to the dam. In contrast, peak brain ChE inhibition in the dam is later following lower repeat exposure (7 mg/kg/day peaks at 5 hours, Lassiter et al. 1998a) compared to a single dose in late gestation (2 hours) at high doses (50 mg/kg/day, Ashry et al. 1992).

Tables 5-7 provide summary information from AChE studies in gestational and post-natal studies in rats. More detailed versions of these tables can be found in Appendix B. The information provided here focuses on effects at or near a dose of 1-1.5 mg/kg. This dose has been used by numerous investigators evaluating both AChE inhibition and other toxicities. As such this dose provides a comparison point for comparing among studies, different toxicities, duration of exposure, ages, lifestages, and methods of administration. Comparisons across different studies need to be made with care as timing of sampling varies among studies which impacts results.

3.2.1. Gestational exposure

In gestational studies with chlorpyrifos, AChE activity is generally inhibited more in dams than in the fetus (Table 5). A similar pattern has been seen for many other OPs (USEPA, 2006, Attachment 1). As such, it would appear that the fetus may be protected by the dam. However, rat fetal brain ChE activity increases 4.3 times from GD14 to GD18 and that activity increases another 3 times from GD18 to PND1. According to Lassiter et al (1998a), "new synthesis of uninhibited cholinesterase

molecules may dilute the inhibited molecules such that the fetal brain cholinesterase activity recovers more quickly than the maternal brain.” Therefore, at a given time after exposure, cholinesterase may appear less inhibited by chlorpyrifos in the fetus compared to adults because the fetus recovers more quickly by rapidly synthesizing new brain cholinesterase. Further support for Lassiter’s comments are found in TK studies mentioned above. Following gestational exposure to the dam, Hunter et al. (1999) found that levels of TCP in the fetal brain were 2-3 times higher than in the maternal brain. Additional data are found in Mattsson et al (1998, 2000) who showed that chlorpyrifos levels were 2-fold higher in maternal blood than fetal blood but TCP levels were similar. Thus, when the dam is exposed to chlorpyrifos, the fetus is as well-likely at similar levels. As such, although the AChE data consistently shows more inhibition in the dam compared with the fetus, the fetus may not actually be protected by the dam. Therefore, AChE data in fetuses from repeated dosing gestational studies may not accurately reflect potential fetal toxicity at a particular dose.

Table 5. Summary of repeated studies evaluating gestational exposure to maternal rats and fetuses.

Study	Route (vehicle)	Time of exposure	Time of measurement post-dosing	Dose	Fetal inhibition	Maternal inhibition	Compartment
Mattsson et al. (1998, 2000)	oral gavage (corn oil)	GD6-20	4 hrs	1 mg/kg/day	8% (NS)	10%/7% (p<0.02)	forebrain
					0%	12%/7% (NS)	hindbrain
					5% (NS)	87%/85% (p<0.02)	RBC
					4% (NS)	77%/60% (p<0.02)	plasma
					0%	49%/50% (p<0.02)	heart
Hoberman et al. 1998a,b, Maurissen et al (2000)	oral gavage (corn oil)	GD6-20	4-5 hrs	1 mg/kg/day	N/A	68.9%	plasma
					N/A	84.4%	RBC
					N/A	17.9%	brain
Mattsson et al. 1998, (2000)	oral gavage (corn oil)	GD6-PND1	2 hrs	1 mg/kg/day	5% (NS)	6% (NS)	forebrain
					0%	6% (NS)	hindbrain
					0%	90% (p<0.02)	RBC
					5% (NS)	80% (p<0.02)	plasma
					2% (NS)	40% (p<0.02)	heart
Qiao et al. (2002)	s.c. injection (DMSO)	GD 17-20	GD 21	1 mg/kg/day	3% (NS)	brainstem	N/A
					6% (NS)	forebrain	N/A

3.2.2. Post-natal, acute exposures

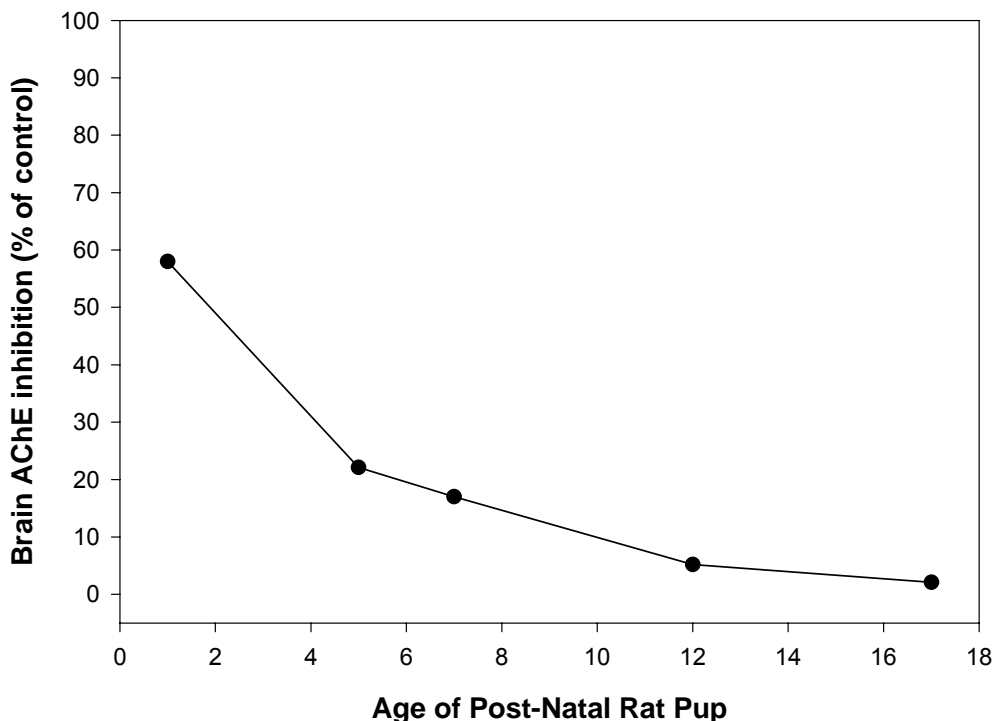
In post-natal studies where pups are directly exposed, the degree of ChE inhibition is clearly age dependant following single exposures (Table 6, Figure 3). In general, blood and peripheral measures are more inhibited at the same dose compared with brain measures. As mentioned in the metabolism section, newborn and juvenile rats are more sensitive to AChE inhibition caused by chlorpyrifos than adult rodents, not because of a difference in the affinity of chlorpyrifos oxon to AChE, but because maturation of detoxification enzymes (Iyer, 2001). This ontogeny (and resulting reduced sensitivity) is evident in Figure 3 where the degree of brain AChE inhibition decreases with the age of the post-natal pup.

Table 6. Summary of acute studies evaluating post-natal exposure to juvenile rats.

Study	Route (vehicle)	Age	Time of measurement post-dosing ^a	Dose	Inhibition	Compartment
Dam et al. (2000)	s.c. injection (DMSO)	PND 1	2 hrs	1 mg/kg	70% (M); 25% (F)	brainstem
					80% (M); 10% (F)	cerebellum
					60% (M); 35% (F)	forebrain
Betancourt and Carr (2004)	oral gavage (corn oil)	PND 1	12 hrs	1.5 mg/kg	58%	forebrain
Timchalk et al. (2006)	oral gavage	PND 5	3 hrs	1 mg/kg	22.1%	brain
					45.7%	RBC
					62.1%	plasma
Zheng et al, 2000	oral gavage (peanut oil)	PND 7	4 hrs	1.5 mg/kg	17%	frontal cortex
					32%	RBC
					51%	plasma
Timchalk et al. (2006)	oral gavage	PND 12	6 hrs	1 mg/kg	5.2%	brain
					27.0%	RBC
					33.4%	plasma
Timchalk et al. (2006)	oral gavage	PND 17	24 hrs	1 mg/kg	2.1%	brain
					15%	RBC
					21.9%	plasma
Moser et al. 2006	oral gavage (corn oil)	PND17	4.5 hrs	0.5 mg/kg	0%	brain
				2 mg/kg	10%	
				0.5 mg/kg	10%	whole blood
				2 mg/kg	40%	

a. Reported time of peak effect

Figure 3. Plot of brain AChE inhibition in post-natal pups following a single dose of 1 mg/kg



3.2.3. Post-natal, repeated exposures

Table 7 and Figure 4 summarize information from repeated dosing studies in post-natal pups using a dose of 1-1.5 mg/kg/day as a point of comparison. Repeated dosing studies show similar degrees of brain AChE inhibition independent of duration of exposure. For example, Guo-Ross et al (2007) and Richard and Chambers (2005) each measured similar amounts of brain AChE inhibition but Guo-Ross et al (2007) dosed pups with only 4 exposures whereas Richard and Chambers (2005) used 6 exposures. The pattern of similar degrees of AChE inhibition across repeated dosing post-natal studies likely reflects the rapid nature of AChE recovery observed by multiple investigators (Chakraborti *et al.*, 1993; Moser and Padilla, 1998; Pope et al., 1991; Pope and Liu, 1997). This pattern is less evident at higher doses where AChE inhibition has reached >70-80% and/or where metabolic processes may be saturated.

One exception to this is the PND1-11 group in Betancourt and Carr (2004) where no brain AChE inhibition was reported. When evaluating the results within the Betancourt and Carr (2004) study, there is a decrease in inhibition following repeated dosing studies from PND1-3, 1-6, and 1-11 suggesting that as the pups mature, they become less sensitive. A similar but less pronounced trend was observed by Richards and Chambers (2005) who showed that PND1-6 and 1-12 dosing resulted in similar degrees of inhibition. In the PND1-21 group, a somewhat lower amount of brain AChE

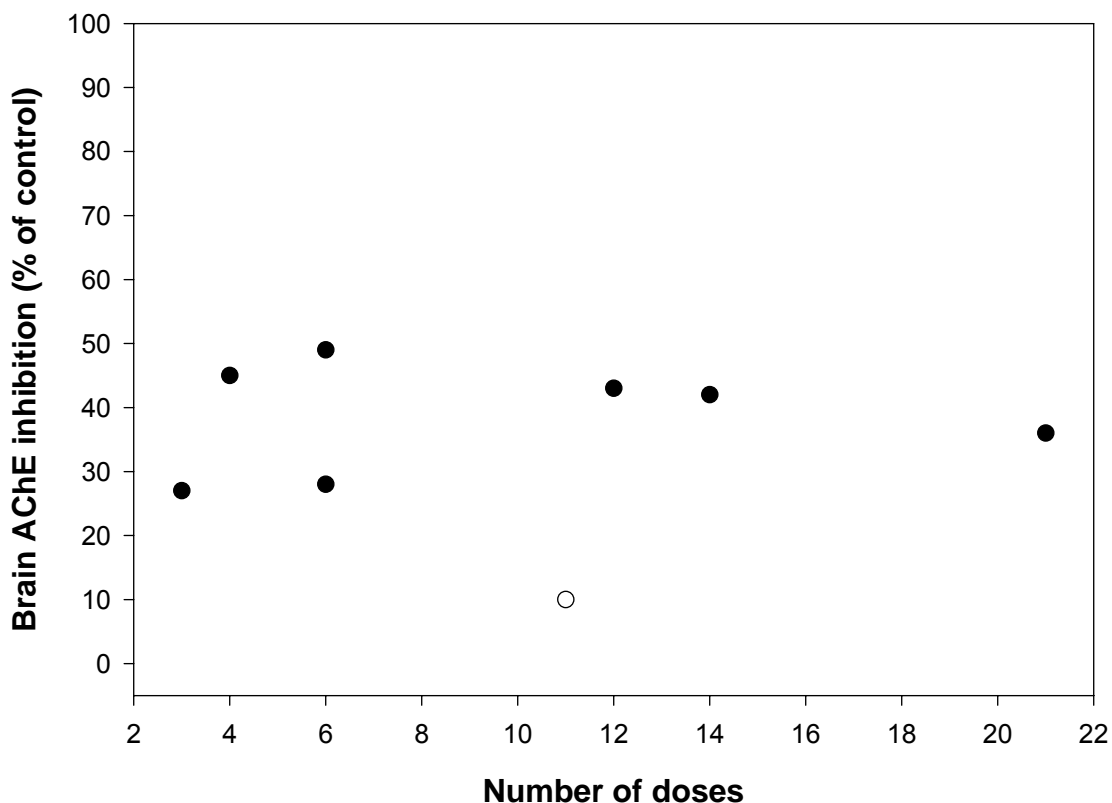
inhibition was observed (36%) compared with the PND1-6 and 1-12 groups. There are potential explanations for this. First, the results of Richards and Chambers (2005) may be explained based on the timing of measurement, PND1-6 animals were measured at 6 hours post-dosing but the PND 1-21 was measured 24 hours post-dosing. Alternatively, the reduced brain AChE inhibition could have resulted from maturation of detoxification pathways resulting in decreased inhibition.

It is notable that the trend shown in Figure 4 is distinctly different from results in adult studies for most OPs. Typically in adult rats, AChE inhibition increases with repeated exposures. In other words, at a common dose level, more inhibition is observed after repeated exposures compared with a single exposure.

Table 7. Summary of repeated studies evaluating post-natal exposure to juvenile rats.

Study	Route (vehicle)	Time of exposure	Time of measurement post-dosing	Dose	Inhibition	Compartment
Guo-Ross et al. (2007)	oral gavage (corn oil)	PND 1-4	4 hrs	1 mg/kg/day	25%	brain
				1.5 mg/kg/day	45%	
Betancourt and Carr (2004)	oral gavage	PND 1-3	24 hrs	1.5 mg/kg/day	27%	forebrain
Song et al. (1997)	s.c. injection (DMSO)	PND 1-4	24 hrs	1 mg/kg/day	24%	brainstem
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1-6	6 hrs	1.5 mg/kg/day	49%	brain (excluding cerebellum and medulla-pons)
Betancourt and Carr (2004)	oral gavage	PND 1-6	24 hrs	1.5 mg/kg/day	28%	forebrain
Betancourt and Carr (2004)	oral gavage	PND1-11	24 hrs	1.5 mg/kg/day	None	forebrain
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1- 12	12 hrs	1.5 mg/kg/day	43%	brain (excluding cerebellum and medulla-pons)
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1-21	24 hrs	1.5 mg/kg/day	36%	brain (excluding cerebellum and medulla-pons)
Zheng et al (2000)	oral gavage (peanut oil)	PND7-20	4 hrs	1.5 mg/kg/day	42%	frontal cortex
					57%	RBC
					59%	plasma

Figure 4. Plot of brain AChE inhibition in post-natal pups following repeated dosing at 1.5 mg/kg



3.2.4. Method of administration

AChE studies available for chlorpyrifos use a variety of methods of administration. The two most common are oral gavage and subcutaneous injection, particularly with DMSO. Some have suggested that the TK properties of a particular chemical may vary by method of administration, thereby impacting the amount of AChE inhibition observed in a particular study. However, the Agency's analysis suggests that the inhibition levels may be more similar than previously believed.

In general, study designs in gestational studies vary widely among laboratories with regard to doses used, number of repeated doses, and gestational days of dosing which makes comparing the results problematic. Chanda and Pope (1996) exposed dams from GD 12-19 via subcutaneous injection with peanut oil and showed 75% brain inhibition 24 hours after the last dose in the dams at a dose of 6.25 mg/kg/day. Hunter et al (1999) and Lassiter et al (1998a) exposed dams using corn oil gavage from GD14-18 at 7 mg/kg/day and observed 68% and 69% brain AChE inhibition 24 hours after the last dose, respectively. The gestational days of dosing differs between the studies and the number of doses differs between the studies--8 and 5 for Chanda and Pope (1996)

and the EPA studies (Hunter et al, 1999; Lassiter et al, 1998a), respectively. Even when considering the differences in study designs, there is notable similarity in the amount of measured brain AChE inhibition at 24 hours after the last dose in the studies using subcutaneous injection and oral gavage—75% and 68-69% (Chanda and Pope, 1996; Hunter et al, 1999; Lassiter et al, 1998a, respectively).

Comparison of post-natal studies show that brain AChE inhibition at similar dose levels (e.g., Tables 5 and 6) yields remarkably similar results in young pups (ages PND1-5). For example following an acute dose of 1 or 1.5 mg/kg/day in PND1 pups, 60% and 58% forebrain AChE inhibition were noted from subcutaneous injection with DMSO and corn oil gavage, respectively (Dam et al, 2000; Betacourt and Carr, 2004). Following exposure at 1 mg/kg/day from PND1 to PND4, 24% and 25% brain AChE inhibition were noted from subcutaneous injection with DMSO and corn oil gavage, respectively (Song et al, 1997; Guo-Ross et al, 2007). The time measurements of these studies were at 24 hour and 4 hours post-dosing for subcutaneous injection and gavage, respectively. The Agency also notes that preliminary (not yet replicated or published by the authors) data by Carr and Narr presented at SOT (2008) showed striking similarity in the time course and amount of brain AChE inhibition in PND10 pups exposed at 5 mg/kg from subcutaneous injection with DMSO and corn oil gavage. Moreover, the amount of brain AChE (25-28%) in the Carr poster is similar to that PND11 pups exposed at the same dose from Dam et al (2000) who used subcutaneous injection (15-30% brain stem, but 15-35% for brain) at 4 hours post-dosing.

A recent study Marty et al (2007) provides TK data which supports findings of AChE studies mentioned above. Specifically, Marty et al (2007) compared methods of administration for PND5 pups exposed to 1 mg/kg/day chlorpyrifos via corn oil gavage, subcutaneous injection with DMSO, and oral exposure in milk. Across the three methods of administration, Marty et al (2007) showed relatively small (2-fold or less) differences in: 1) AUC for chlorpyrifos and TCP; 2) $\frac{1}{2}$ lives for TCP; and 3) similar time to peak effect for chlorpyrifos and TCP. Based on the findings of Marty et al (2007), there appear to be only small differences in TK characteristics in PND5 pups exposed via corn oil gavage, subcutaneous injection with DMSO, and exposure in milk.

The Agency has concluded for young pups, at least up to post-natal day 5 in rat, that administration via the oral route and subcutaneous injection provide remarkably similar results and that post-natal studies up to PND 5 in either route are relevant for risk assessment. Less data are available to compare routes/methods of administration for older pups and no comparative TK data are available for gestational exposures. As more studies are available in the future, the Agency may, if appropriate, extend this conclusion to include older pups. The lack of comparative PK for oral gavage and subcutaneous injection in pregnant dams and fetuses is considered an important data gap in quantitatively evaluating dose response data in subcutaneous injection studies. However, the Agency can not discount the findings of subcutaneous injection gestational studies at this time.

3.2.5. Preliminary conclusions

Numerous AChE studies are available in different lifestages and ages in rats. These studies vary widely by the level and number of doses used, availability of time course information, and method of administration. The Agency has preliminarily concluded the following

- Repeated dosing gestational studies which show less fetal brain AChE inhibition compared with the dam may not reflect actual toxicity to the pup. This conclusion is based, in large part, on TK data comparing blood and brain levels of chlorpyrifos and/or its metabolites in fetal and dam tissues.
- Following acute post-natal exposure studies, there is an age-dependant sensitivity that decreases as the pups mature.
- When considering the repeated dosing post-natal studies across laboratories, there is little variability with respect to degree of brain AChE inhibition across different durations of exposure. Within a laboratory, however, decreases in sensitivity have been observed with longer duration of exposure.

The Agency will solicit comment on each of these preliminary conclusions and the science which does and does not support them.

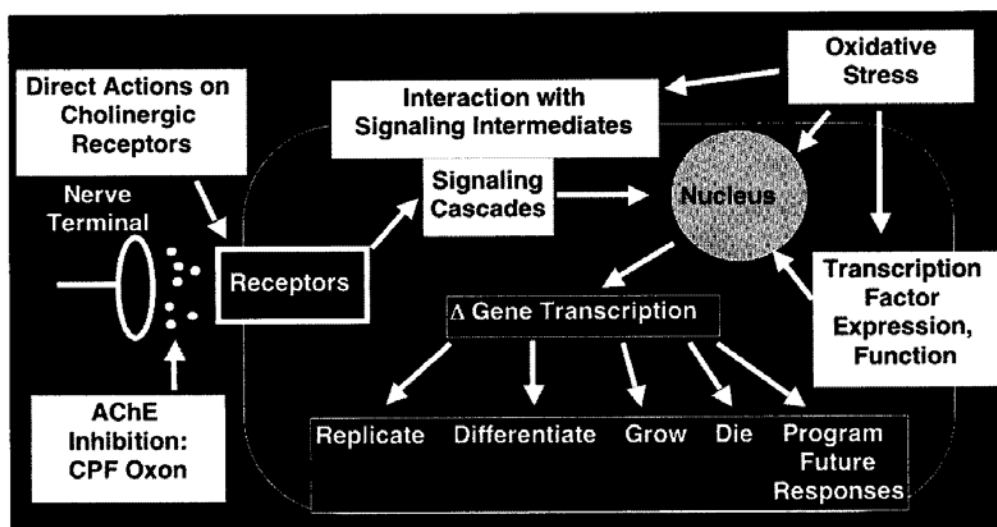
3.3. Effects on the Developing Nervous System

There is a growing body of literature on the effects of chlorpyrifos in the developing brain which indicate that gestational and early postnatal exposure can lead to neurochemical and behavioral alterations into adulthood; these changes are observed long after potential AChE inhibition has recovered. Indeed, some authors report finding no or only marginal inhibition of fetal or neonatal brain AChE at the doses causing such effects. Figure 5 provides a schematic of some of the mechanisms or toxic pathways that have been proposed for chlorpyrifos (Slotkin, 2006). One such hypothesis involves the morphogenic role of AChE, such that inhibition may adversely impact the development of the central and peripheral nervous systems to cause permanent damage (Brimijoin and Koenigsberger, 1999 and Bigbee et al, 1999). Although multiple mechanisms have been proposed (described in Appendix C), a coherent mode of action with supportable key events, particularly with regard to dose-response and temporal concordance, has not yet been elucidated.

Many recent studies elaborate on the alterations induced by peri-natal chlorpyrifos exposure (see Appendix C). The results of these studies contribute to the overall hazard characterization of chlorpyrifos and are important in on-going efforts to define pathways of toxicity for chlorpyrifos. In this section, however, the Agency has focused on studies evaluating behavioral measures following gestational and/or postnatal exposure to chlorpyrifos. Behavioral measures are available from multiple laboratories and in two species. Although some differences among the studies have been observed, the behavioral outcomes are generally reliable, and are particularly valuable as they show

qualitative similarities to some neurodevelopmental findings reported in children (described in Section 2.D below).

Figure 5. Multiple possible mechanisms of chlorpyrifos (From Slotkin 2006)



3.3.1. Behavioral Effects in Rats

A series of recent behavioral studies from a single laboratory (Drs. Slotkin, Levin, and co-workers) have described a variety of effects in adult Sprague-Dawley rats that were treated with chlorpyrifos during several different periods of development: early gestation (GD 9-12; Icenogle et al 2004); late gestation (GD 17-20; Levin et al, 2002) or after birth on PND 1-4 (Aldridge et al., 2005c; Levin et al., 2001) or PND 11-14 (Levin et al, 2001). Chlorpyrifos, either 1 and/or 5 mg/kg, was administered subcutaneously in DMSO. Exposures took place either during gestation (GD 9-12 or GD 17-20) or else after birth (PND 1-4 or PND 11-14), and behavioral testing was initiated at approximately 4 weeks of age, continuing for several months. These studies are summarized in Table 8. AChE inhibition was not directly measured in any of these studies.

Table 8. Effects following gestational or postnatal exposure to 1 or 5 mg/kg chlorpyrifos administered subcutaneously in DMSO

Reference	Ice-nogle (2004)	Levin (2002)	Levin (2001)	Aldridge (2005)	Levin (2001)
Window	GD 9-12	GD 17-20	PND 1-4	PND 1-4	PND 11-14
Dose	1 or 5 mg/kg ^(a)	1 or 5 mg/kg ^(b)	1 mg/kg	1 mg/kg	5 mg/kg
Assessment PND	~4-17 weeks	~4-17 weeks	~4-17 weeks	~7-17 weeks	~4-17 weeks
Chocolate Milk Preference	-- ^(c)	--	--	♂/♀ ↓ preference	--
Elevated Plus maze	↑center crosses	--	--	♂ ↑ time in open arms & ↑center crosses	--
Motor Activity	↑ habituation rate	↓ ♀ habituation rate	No effect	--	♂/♀ ↓ habituation rate
Radial arm maze (RAM) working & reference memory	↑ errors early in training	♀ ↑ errors early in training	♀ ↓ errors throughout, ♂ ↑ errors early in training	♀ ↓ errors throughout, ♂ ↑ errors early in training	No effect
T-maze activity & alternation	↓ activity early in session	↓ activity early in session	♂ ↓ activity middle of session	--	♂ ↓ activity middle of session
Startle reflex & prepulse inhibition	No effect	--	--	--	--
Ketanserin Challenge RAM	--	--	--	♂/♀ ↑ errors	--
Scopolamine Challenge RAM	↓ scopolamine effect	♀ ↓ scopolamine effect	No effect	--	♀ ↓ scopolamine effect
Mecamylamine Challenge RAM	No effect	No effect	No effect	--	No effect

^(a) No statistical interaction of treatment and sex, therefore sexes were combined for analyses. Data are for the 5 mg/kg dose group which was most affected, 1 mg/kg dose group had little if any effect except in the T maze; ^(b) Effects significant only at 1 mg/kg dose for RAM, both doses for T maze and motor activity; ^(c) -- not tested.

Cognitive function has been repeatedly tested in these studies, using both a T maze to measure spontaneous alternation (a crude form of learning) and the radial arm maze (RAM). The RAM evaluates both working (short-term) and reference (long-term) memory throughout several weeks of training. As shown in Table 8, adult rats treated with chlorpyrifos during gestation or early lactational periods showed altered learning on both forms of memory; only when exposure occurred on PND 11-14 was there no effect of treatment. In general, chlorpyrifos-treated rats displayed more errors early in the training sessions, but were able to learn the task eventually. Gender differences in this pattern of response emerged but were dependent on the period of exposure – the effect was seen in GD 17-20 females, but PND 1-4 males. On the other hand, female rats treated PND 1-4 displayed fewer errors than controls throughout the training session. The authors have interpreted this counterintuitive result as indication of an attenuation of normal gender differences, since the treated female rats showed the same error rate as males, or else possibly an enhancement of cholinergic response (Levin et al., 2001; Aldridge et al., 2005a,b,c). It is interesting to note that spontaneous alternation was not altered in any of the studies.

The involvement of underlying neurotransmitter systems may be delineated by using pharmacological challenges. After RAM training, rats were administered scopolamine (a muscarinic antagonist which produces acute cognitive deficits), mecamylamine (a nicotinic antagonist which by itself does not alter cognitive function), and ketanserin (a serotonergic antagonist, which does not alter cognitive function). In most cases, the normal amnesic effect of scopolamine was blocked in the rats treated gestationally or in later lactation, but not in the PND 1-4 groups. Mecamylamine had no effect in any groups in any study. On the other hand, chlorpyrifos-treated rats showed a dose-related increase of errors when challenged with ketanserin, which had no effect in controls. Taken together, these data indicate that the muscarinic cholinergic system may be sub-functional as a result of treatment, and these effects are specific for gender and window of exposure. In addition, the serotonergic system may be playing an abnormal role to enable cognitive abilities. Thus, the authors' interpretations over these studies are that there is a wide window of vulnerability to chlorpyrifos ranging from early gestation (neurulation) to late lactation that can impair cholinergic circuits used in learning and memory (Aldridge et al., 2005a,b,c).

Other chlorpyrifos effects on the serotonergic nervous system were evaluated using other behavioral tests. Treated rats showed alterations in preference for a preferred substance (chocolate milk) and increased time in the open arms of an elevated plus maze (which evaluates anxiety). These effects, along with the abnormal responsiveness to ketanserin in the RAM, suggest long-term differences in this system following early postnatal exposures. These behavioral effects are concordant with ongoing neurochemical studies from the same laboratory, as described in Appendix C.

Gender-selective deficits in locomotor development are also reported in several of these studies, where most evaluations were conducted within a couple of weeks of exposure (starting at 4 weeks of age). Activity levels recorded as latencies in the T maze were decreased in the early portions of the test sessions in gestationally exposed rats. Marginal decreases were observed in PND 1-4 male rats, but only in the middle of training. Overall activity measured in a figure-8 maze was never altered, but the rate of habituation (the change in activity during the test session) was either increased or decreased, depending on gender and window of exposure. An earlier study from the same laboratory (Dam et al., 2000) also reported activity changes at 4 weeks of age, but in this case, only males showed decreased open-field activity and rearing following exposure to 1 mg/kg on PND 1-4. Using a different dosing paradigm, Carr et al. (2001) did not find open-field activity changes in rats receiving 3 mg/kg/day from PND 1-21, but when the doses were escalated (up to 6 mg/kg/day or 12 mg/kg/day) decreases were observed 1-2 weeks after exposure ended. There is an indication of long-term changes in activity in that the number of center crosses was increased in the elevated plus maze in both studies where it was evaluated, and this test took place after RAM training. On the other hand, however, latencies in the RAM were never altered by chlorpyrifos. While it appears that there is little consistency in these findings, it is important to note some differences in the procedures and apparatus in which activity was measured.

There is only one other study of which we are aware which involved short-term developmental dosing with chlorpyrifos, followed by a test of learning and memory. Jett and coworkers (2001) used a Morris water maze, which evaluates spatial learning and memory, to test adolescent (24-28 day old) Long-Evans rats. One group received subcutaneous injections of chlorpyrifos (peanut oil vehicle) at 0.3 or 7 mg/kg on PND 7, 11, and 15, the other on PND 22 and 26. Only the high dose slowed learning in the earlier postnatal exposure group, whereas both doses were effective in the postweaning exposure group. There was no brain AChE inhibition in comparably treated rats sacrificed on PNDs 7, 8, 16, or 28. This dosing regimen is unique to this study and not comparable to the other studies described above. Since dosing occurred either shortly before or during testing, the results may be confounded and reflect acute toxicity of chlorpyrifos. These results have not been repeated, nor has testing been conducted at later times after exposure using this paradigm.

It is important to note that the guideline developmental neurotoxicity study includes measures of locomotor activity as well as learning and memory, with assessments occurring at weaning and again at 2 months of age. In the chlorpyrifos DNT study conducted by the manufacturer (Maurissen et al., 2000), dams were dosed from GD 6 to PND 11. There were changes in the startle response (decreased amplitude and increased latency) in PND22 rats, as well as a suggestion (non-significant) of increased activity levels in adult offspring at the high dose, 5 mg/kg/day; however, this dose level was maternally and developmentally toxic. No significant changes were reported using a T maze delayed alternation task at either age.

3.3.2. Behavioral Effects in Mice

Persistent effects of chlorpyrifos following gestational and/or early postnatal exposure were also demonstrated in CD-1 mice in a series of studies from the laboratory of Dr. Calamandrei. One study involved subcutaneous administration in DMSO on either PND 1-4 or 11-14 (Ricceri et al., 2003), while the others included both oral administration of the dams on GD 15-18 followed by dosing of the pups on PND 11-14 (Ricceri et al., 2006; Venerosi et al., 2006). The combination of pre- and postnatal exposures renders interpretation more difficult, and less comparable to the studies in rats.

Ricceri et al (2003) included assessment of brain AChE activity after exposure on PND 1-4 or PND 11-14 and report inhibition only at 1 hr (but not later times) after dosing on PND 4 (little difference between the dose groups). There was no effect in the mice dosed on PND 11-14. The Agency notes that mice may be less sensitive than to rats to chlorpyrifos (USEPA, 2000), yet the dose of 1 mg/kg/day was sufficient to produce significant inhibition in the young pups. AChE activity measured in a later study (Ricceri et al., 2006) also reported no brain inhibition in pups dosed PND 11-14, but serum activity was lowered (to approximately 50% control levels) in both postnatal chlorpyrifos groups.

Ricceri and colleagues (2003) measured a variety of social, motor, and cognitive behaviors before weaning and up until PND 60. Notable persistent behavioral effects were apparent and included increased open-field locomotor activity (on PND 25, in the PND 11-14 group mice only, both doses), more activity in a novel environment (on PND 35, all treatment groups), and more aggressive responses by males in the social interaction test (PND 45, all treatment groups). On PND 60, passive learning, however, was not affected.

In later studies by the same group (Ricceri et al., 2006; Venerosi et al, 2006), exposure occurred during GD 15-18 at 3 or 6 mg/kg chlorpyrifos (oral administration to the dam), followed by exposure of the offspring on PND 11-14 at a dose of 1 or 3 mg/kg chlorpyrifos (subcutaneous administration in peanut oil). Focusing on the mice that received vehicle first, then chlorpyrifos, or vice versa, allows evaluation of treatment effects pre- or postnatally. Ricceri et al. (2006) reported that, when tested as adults, motor activity was increased in mice that received the high dose during GD 15-18. Postnatal exposure increased male agonistic behaviors in the high-dose group (supporting their finding from 2003), and induced-maternal behaviors in both dose groups. Postnatally treated females also spent more time in the open arms of an elevated plus maze. In another study of adult females (Venerosi et al., 2006), the authors reported that while chlorpyrifos exposure during gestation altered social behavior toward the same and new partners, these outcomes were not observed when the mice also received chlorpyrifos postnatally. Such potential compensation of gestational exposure requires additional study.

3.3.3. Discussion

In general, studies in the literature have used doses of 1 to 6 mg/kg/day chlorpyrifos, during gestation and/or lactation. Exceptions are the Jett study (Jett et al., 2001) and the manufacturer's developmental neurotoxicity study (Maurissen et al., 2000), both of which used 0.3 mg/kg/day as the lowest dose. While the latter detected no changes at that lowest dose, the former reported changes in water maze learning that took place shortly after or during dosing. The studies from the Slotkin/Levin and Calamandrei laboratories provide evidence that adults may exhibit persistent behavioral changes following peri-natal exposures. Since both laboratories included a dose of 1 mg/kg/day, some comparisons in response may be made – these are summarized in Table 3 (Section 2.0). While the precise pattern and direction of changes are not always consistent, this is not totally unexpected given the many experimental factors that are different, including window of exposure, age at testing, specificity of behavior measured, and many others. When evaluated as a whole, however, these studies provide a basis for concern for susceptibility for persistent effects of chlorpyrifos as low as 1 mg/kg/day on neurodevelopment.

In conclusion, there is a growing body of literature with laboratory animals indicating that gestational and/or early postnatal exposure to chlorpyrifos may cause persistent behavioral effects into adulthood. There are also concurrent changes in brain neurochemistry based on both *in vivo* and *in vitro* studies that may underlie these behavioral changes into adulthood. The cholinergic nervous system is a target for chlorpyrifos, through AChE inhibition associated with acetylcholine destruction at the synapse and the morphogenic role of this enzyme, as well as possible effects on transporters and receptors. Although there are suggestions for targets for chlorpyrifos other than on the cholinergic system, there is no conclusive evidence identifying them at this time. Specifically, it is still difficult to definitely discern the initiation of the neurochemical changes (i.e., serotonin, macromolecules, etc) reported from concurrent catalytic functional AChE inhibition or an effect on a morphogenic function of AChE. This inability to compare is usually due to study design issues and/or lack of time course information and/or pharmacokinetic data on tissue dosimetry. The studies do, however, provide qualitative descriptions of how the developing rodent brain has a susceptibility to chlorpyrifos exposure with consequences lasting beyond the duration of any observable AChE inhibition and into adulthood.

3.4. Human Epidemiology: Observations in Children

Three major prospective epidemiology cohort studies are looking at pre- and post-natal pesticide exposure in minority mothers and infants, birth outcomes, genetic susceptibility plus long-term childhood neurobehavioral and neurodevelopment outcomes. Funded by multiple Federal Agencies, including US EPA, the study sites are: (1) Columbia University, NYC, (2) Mt Sinai, School of Medicine, NYC, both with multi-ethnic urban poor women and infants, and (3) University of California at Berkeley (Center for Health Assessment of Mothers and Children of Salinas, CHAMACOS) with

women and their children from farm worker populations. These studies and their reported findings are described in detail in Appendix D.

The following text focuses on outcomes related to mental and cognitive function. Birth outcomes (e.g., birth weight, birth length, head circumference) have been reported by some studies (Whyatt et al. 2003, Berkowitz et al. 2004). However, the type of birth outcome and direction of the change (i.e., increase vs. decrease), vary among the three cohorts. For example, one group (Mt. Sinai) reported decreased head circumference (albeit small) when maternal urinary TCP levels were above the limit of detection (>11 ug/L) and PON1 status was considered (Berkowitz et al. 2004). In contrast, increases in head circumference were associated with increasing maternal urinary DAPs in the CHAMACOS cohort (Eskenazi et al. 2004) and the Columbia team reported no changes in head circumference (Whyatt et al. 2004). The Agency does not discount the reported birth outcomes. Instead, the Agency has elected to emphasize those outcomes which have been replicated by multiple cohorts. Furthermore, as described above, there are multiple animal studies which support the concept that gestational exposure can result in effects on behavior which persist beyond any initial AChE inhibition. Thus, when the animal and epidemiology studies are considered in combination, there is strong evidence from multiple human and animal studies regarding the potential for neurodevelopmental effects of chlorpyrifos.

It is unusual for the Agency to have data from three large, prospective cohorts for consideration in human health risk assessment. Each provides unique and somewhat complementary information:

- The Columbia University NYC cohort includes predominately African American and Dominican women and children. This team has reported indoor air, maternal and cord blood measures of parent chlorpyrifos, and multiple birth and neurodevelopmental outcomes. This cohort was exposed during pregnancy to chlorpyrifos and other pesticides indoors and in food. One focus of the publications from this group involves comparisons between pre- and post cancellation of indoor uses of chlorpyrifos.
- Mount Sinai NYC cohort includes women and children who are Puerto Rican Hispanic, African American, and Caucasian. This team has associated urinary metabolites (TCP and/or DAPs) with some birth and neurodevelopmental outcomes. This group has placed an emphasis on relating outcome information with PON1 status. The enrollment of the Mt. Sinai cohort overlapped with the cancellation of residential uses of chlorpyrifos. However, the researchers have not evaluated the impacts of the residential phase out on the health outcomes measured in their publications.
- The CHAMACOS cohort includes mothers and children from farm families who live in the Salinas Valley, California and who are predominately of Mexican descent. This cohort is exposed to many pesticides, including multiple OPs (Table A-1 in Appendix D), from multiple pathways such as occupational exposures and take-

home exposures. This team has collected information on PON1 status but has not yet published findings associating PON1 status with health outcomes. This team has, however, associated urinary DAP metabolites with some birth and neurodevelopmental outcomes.

These studies have been performed using prospective epidemiology methods such that exposure measures come before the potential outcomes. Chemical measures of blood and urine pesticide analytes are done by, or with, testing methods of the Centers Disease Control and Prevention (CDC) in ways that can be compared to reference ranges in the National Health and Nutrition Examination Survey (NHANES) for DAPs and/or TCP in urine and chlorpyrifos in blood (Columbia only). All three studies used well developed neurodevelopmental measures, which provide comparability. Both the Columbia and CHAMACOS cohorts used the standardized Bayley Scales of Infant Development II (BSID-II), a widely used, normative value-referenced, developmental test for young children that is used frequently to diagnose developmental delay and is known to be highly sensitive to low level intrauterine exposures. All three also used the Child Behavior Checklist (CBCL) to assess behavior problems such as attention problems, attention deficit hyperactivity disorder (ADHD), and pervasive developmental disorder (PDD) problems. The Mt. Sinai results for Bayley Mental Development Index (MDI) and the Bayley Psychomotor Development Index (PDI) are still in preparation (Engel et al., in prep).

In an effort to characterize the exposures and outcomes in the epidemiology studies, the Agency developed a series of detailed tables which compare blood levels of chlorpyrifos and urine levels of TCP in humans and animals across studies and compared these levels with AChE inhibition data where possible. These tables are found in Appendix D. Due to differences in study design between epidemiology and laboratory studies, the interpretation of these tables is challenging and problematic. In animal or human laboratory studies, time course studies provide valuable information such as the time of maximum effect and/or maximal blood or tissue levels and time to recovery. In contrast, in epidemiology studies, the timing of exposure is unknown and thus the timing of measurements in relation to when exposure occurred is also unknown. Specifically, the urine and blood measures in these studies are not timed with applications, so it is difficult to correlate these results with known exposures in the home or agricultural field. The epidemiology studies have taken spot samples of urine (as opposed to 24 hour samples). There is uncertainty associated with spot samples as they may not capture all the pesticide exposures because of the short-half life of the OP pesticides in the body.

Compared with epidemiology studies, laboratory studies, whether animal or human, are highly controlled situations where the amount, timing, and route of exposure are known. In laboratory experiments, important endpoints such as AChE inhibition and behavioral measures or clinical signs can be observed with care. In addition, the magnitude of exposure can be controlled for. Specifically, exposure pathways like food or residential exposures could be controlled for during a laboratory study compared with epidemiology studies where the magnitude of exposure may vary significantly among

individuals and among different days. Similarly, the route of exposure is known for in the laboratory study but is unknown in the epidemiology studies. For example, in the human deliberate dosing studies, the subjects were exposed via the oral route. With regard to the epidemiology studies, mothers were likely exposed through the diet (oral) and from residential uses (dermal, inhalation).

Unlike the birth outcomes which show variable results across the cohorts, delays in mental development were reported in all three cohorts (Columbia, Mt. Sinai and CHAMACOS). Both Mt. Sinai and CHAMACOS cohorts report abnormal reflexes in neonates associated with urinary maternal DAP levels. For each log₁₀ unit increase in total DAPs, these authors report a 32 percent (Engel et al., 2007) and 26 percent (Young et al., 2005) increased risk of abnormal reflexes.

Figure 6. Mental Development Index (MDI) results from CHAMACOS, Mt. Sinai, and Columbia University

	Berkeley (Log ₁₀ DAPs) Adj b	Mt. Sinai (Log ₁₀ DAPs) Adj b	Columbia (High v. Low CPF) Adj b
6 Months	-1.2	--	--
1 Year	-1.3	-1.3	-0.3
2 Years	-3.5**	-1.9**	-1.5
3 Years	--	--	-3.3*

* p <0.1 **p <0.05

Eskenazi *et al.* 2007; Engel *et al.* in preparation; Rauh *et al.* 2006

Source: Rauh 2008, presentation to EPA, April. Used with permission.

Increases in pervasive developmental disorder were reported in both the Columbia and CHAMACOS cohorts. In the Columbia study (Rauh et al. 2006) these effects in 3 year old children were associated with high (>6.17 pg/g) chlorpyrifos blood umbilical cord levels, while the CHAMACOS cohort reported these effects in 2 year old children to be associated with increases in total urinary maternal and child DAPs and DMPs, and child DEPs for 12 month old children (Eskenazi et al. 2007). The Mount Sinai team has not yet published findings for the 2 year old children but these results

are currently under preparation for publication. Based on preliminary information shared with the Agency by the investigators at Mt. Sinai, these results are expected to show increasing urinary DAPs were associated with lower Mental Developmental Index (Engel et al. in prep, Figure 6). Thus, prenatal OP exposure has been reported to be associated with delays in mental development in 2 and 3 year old children, increased odds of abnormal reflexes in neonates, and increased odds of pervasive developmental disorder in children 2 and 3 years of age.

In the CHAMACOS study, however, TCP in maternal urine was not associated with any Bailey or CBCL adverse outcomes in children, and there were no reported associations between PDI or attentional deficits and urinary OP concentrations. It should be recognized that the Columbia study reported effects for 3 yrs olds, while the CHAMACOS study has only published data for 2 yr old children thus far. In addition, the urinary levels of TCP in the CHAMACOS study were much lower (median 3.5 ug/L compared to 7.5 ug/L for the Mt. Sinai cohort) than the Mt. Sinai cohort and this could partially explain the differences in the study results.

The Agency believes that the Columbia University studies provide the most relevant information for evaluating the human health effects of chlorpyrifos. These studies specifically evaluated chlorpyrifos in maternal and umbilical cord blood levels rather than the TCP and/or DAP urinary metabolites in maternal urine reported in the Mount Sinai and CHAMACOS studies. Many OPs can contribute to total urinary DAP concentrations. TCP is a common metabolite of multiple pesticides: chlorpyrifos, chlorpyrifos-methyl and trichloropyr. It is also the primary environmental degradate of chlorpyrifos and is found on food treated with chlorpyrifos. As such, environmental and/or dietary exposures to TCP can also contribute to urinary TCP levels and exposure to multiple OPs complicates interpretation of DAP data.

In the Columbia University cohort, recruitment of study participants overlapped with residential use cancellation; there was a sharp decline in use during this period. Chlorpyrifos levels dropped substantially in maternal personal air and plasma and cord blood plasma samples after cancellation. For children born before the cancellation, 'high' chlorpyrifos exposure in cord plasma was associated with decreased birth weight and length. In contrast, this relationship was no longer significant for newborns born after the cancellation because the blood levels dropped and only one child was in the high group. Likewise, there was no association with chlorpyrifos and neurodevelopmental outcomes after the cancellation, again because all but one of the children had cord blood levels less than 6.17 pg/g.

There were multiple chemical exposures in the Columbia University cohort study, including diazinon and propoxur that are cholinesterase inhibitors, and o-phenylphenol, a disinfectant/fungicide, all of which were measured in 100% of air samples at higher median concentrations than chlorpyrifos. The mean umbilical cord levels were lower than chlorpyrifos (1.1, 3.1 and 4 pg/g for diazinon, 2-isopropoxyphenol and chlorpyrifos, respectively). It is also likely that the diazinon and propoxur metabolites in blood were possibly underestimated because of the relatively short half lives, and the lack of

information to correlate the time of sample collection with pesticide application. The study authors report that after controlling for both diazinon and 2-isopropoxyphenol (metabolite of propoxur) exposure in cord plasma, the associations between birth weight and length and cord plasma (ln)chlorpyrifos remained statistically significant ($p \leq 0.02$) and the effect size remained similar to that seen without 2-isopropoxyphenol in the model (Whyatt et al. 2004). However, a similar analysis was not reported for the neurodevelopmental outcomes, so it is not clear if these chemicals have any influence on the chlorpyrifos findings.

While neurodevelopment deficits may be multifactor in origins, it is not always possible to identify the sources for each case. As discussed previously, these children in cohort are from poor multi-ethnic populations and urban neighborhoods and may experience other health disparities that compound pesticide exposure. Such disparities are linked to health care access, low income and low education, as well as exposure to urban air pollutants. Cicchetti (2007) published a critique of neurodevelopmental outcomes reported by Rauh et al. (2006). Specifically, Dr. Cicchetti commented on the study population socio-demographics like maternal education-and said that Rauh et al. (2006) masked, but did not eliminate educational bias by dichotomizing maternal education into high school graduate or non-graduate. Furthermore, that the “high” and “low” exposure groups differed in their race/ethnicity characteristics which confounds race/ethnicity with exposure, and the finding that more high exposure children had ADHD is meaningless. Dr. Rauh responded to Cicchetti by saying the “high” and “low” groups were clearly defined based on the previous report of reduced birth weight among children with exposure levels above 6.17 pg/g (Whyatt et al. 2004). High school degree was used to adjust for maternal education because the sample was uniformly low income, and thus education was the preferred covariate for social class. Maternal intelligence, although controlled was not significant in their analysis. Rauh et al. (2006) controlled for race/ethnicity in all models and also used a stratified analysis showing a significant chlorpyrifos effect within each ethnic group, independent of race. They used Achenbach’s Child Behavior Checklist (CBCL) to assess behavior problems rather than make a diagnosis because ADHD is hard to diagnose in preschool-aged children.

Cicchetti (2007) also commented on the significance of differences in neurodevelopmental measures and suggested the mean Mental Development Index scores were clinically meaningless, and indicated that there were no standards defining “high” and “low” chlorpyrifos exposure. Rauh et al. (2007) disagrees with Dr. Cicchetti claim that the significant chlorpyrifos effect on Mental Development Index was “clinically meaningless” and indicates that a Bayley developmental score < 85 prompts referral to early intervention services, and exposures that produce small shifts in the mean often result in more children who meet the diagnostic criteria.

The Agency can not rule out the potential for multiple AChE-inhibiting pesticides impacting the health outcomes reported in the children. In the individual chemical risk assessments for all three, indoor residential exposures provided risk estimates above the Agency’s level of concern. As a result, some indoor uses for all three pesticides

have been voluntarily cancelled by registrants⁴. This conclusion regarding multi-chemical exposure does not preclude the potential contribution of chlorpyrifos in the reported health outcomes. Given, that measured levels of chlorpyrifos have been statistically associated with multiple birth and neurodevelopmental outcomes and these blood levels have been correlated in time with the chlorpyrifos phase-out, the Agency has preliminarily concluded that chlorpyrifos likely played a role in these outcomes. The Agency will solicit comment from the panel on the preliminary conclusions with regard to the usefulness of information from each cohort for the chlorpyrifos risk assessment, the associations between chlorpyrifos exposure and health outcomes, and the qualitative similarities noted between the epidemiology studies with animal studies.

3.5. Extrapolation Factors

The following text is a summary of more detailed information which can be found in Appendix E on developing and using DDEFs and the specific evaluation of TK and TD data for chlorpyrifos.

In previous risk assessments, the Agency has applied the default 10X factors for both inter- and intra-species extrapolation in addition to the FQPA 10X safety factor. U.S. and international efforts have made significant efforts to improve the scientific basis for human health risk assessments by increasing the use of mechanistic and kinetic data. One such area is the decreased reliance on default uncertainty factors through the development of Chemical Specific Adjustment Factors (often called Data-Derived Extrapolation Factors, DDEFs). In 2005, the WHO published its guidance for deriving chemical specific adjustment factors (CSAFs; WHO, 2005). The guidance is based in large part on analyses by Renwick (1993) and Renwick and Lazarus (1998) and describes the use of TK and TD data as a means of replacing the traditional 10X safety factors for human sensitivity and experimental animal-to-human extrapolation. EPA has an on-going effort to develop similar guidance and has used these concepts in some risk assessments including several pesticides.

A preferred approach to extrapolate from animals to humans and within humans would be to use a PBPK or other sophisticated model. However, such a model is not currently available for assessment of chlorpyrifos exposure during pregnancy or for young children. In the absence of such a model, extrapolation factors to account for inter- and intra-species variability are used. Such factors based on data are more scientifically robust than use of default factors. The Agency has evaluated the extent to which data are available to develop DDEFs for chlorpyrifos. Given the remaining uncertainty regarding the modes(s) of action affecting the developing brain, the Agency has elected to not develop a DDEF for UF_{AD} or UF_{HD} . As such, the Agency proposes to apply the default 3X for inter- and intra-species TD extrapolation (i.e., UF_{AD} and UF_{HD}).

⁴ Diazinon residential uses were also phased out, with retail sales for indoor uses ceasing by December 2002, a year after chlorpyrifos.

The Agency has evaluated data on P450s, carboxylesterases, BuChE, and PON1 for inter-species TK extrapolation (i.e., UF_{AK}). Due to limited data and based on differences in rat and human pregnancy with regard the timing of maturation of metabolic processes, there are uncertainties surrounding appropriate metabolic parameters for animal to human extrapolation. This uncertainty precludes the development of a DDEF for inter-species TK extrapolation (i.e., UF_{AK}). Thus, the Agency proposes to apply the default 3X for UF_{AK} .

Regarding within human variability, the Agency again evaluated data on P450s, carboxylesterases, BuChE, and PON1. Studies on carboxylesterases and BuChE are limited by number of sample/subjects and/or by lack of data in juveniles. Data on P450s are complicated by multiple enzymes each with its own maturation profile. Others have evaluated the P450 literature for use in derivation of child specific UFs with poor success (Ginsberg et al, 2004a). One of the key detoxification enzymes of chlorpyrifos, paraoxonase 1 (PON1) is an A-esterase which can metabolize chlorpyrifos oxon without inactivating the enzyme (Sultatos and Murphy, 1983). Extensive population variability data from blood are available for PON1.

There are multiple PON1 polymorphisms reported in the literature including two in the coding region, at least 13 in the noncoding region, and more than 150 single nucleotide polymorphisms (snp; Jarvik et al, 2003). The amount of information on each varies widely. The Q/R polymorphism at position 192 results from a Gln/Arg substitution and affects catalytic efficiency (Humbert et al, 1993; Adkins, et al, 1993; Blatter Garin et al, 1997; Mackness et al, 1998). Specific to activity on chlorpyrifos oxon, the R192 alloform has a higher catalytic efficiency of hydrolysis compared to the Q192 alloform (Cole et al, 2005). This would suggest that individuals with the Q192 alloform may be more sensitive. In the preliminary analysis, the Agency has focused on the PON1-192 polymorphism since it has been studied more extensively than any other, has been linked to chlorpyrifos oxon sensitivity in animal studies, and has been evaluated in studies attempting to associate PON1 status with health outcome following OP pesticide exposure in adults and children.

The analysis summarized in Table 9 is *preliminary*. The Agency will be soliciting comment from the SAP on several aspects of the current analysis. As described in detail in Appendix E, the Agency has followed the 2005 IPCS guidance. In that guidance, UF_{HK} can be determined as the ratio of the dose metric at a lower percentile (e.g., 10th, 5th, 2.5th, 1st percentile of the distribution) for those deemed sensitive and a central tendency measure of the general population. The 50th and 5th percentiles were calculated for each genotype and/or age group. Ratios of the 50th-percentile and the 5th-percentile were calculated. The Agency has performed calculations on the QQ, QR, and RR genotypes but has only reported the results for the QQ and QR genotypes here as these groups are potentially more sensitive to chlorpyrifos or its oxon (Holland et al, 2006; Cole et al, 2005; Furlong et al, 2005). Data on paraoxonase (POase), ARase, and CPOase have been evaluated. There are two alternatives to performing the calculations: 1) compare the 5th percentile of the QQ group to the 50th percentile of the QQ group 2) or to compare the 5th percentile of the QQ group to the 50th percentile of

the QR group. The QR would, theoretically, represent the intermediate-speed metabolizers and potentially more representative of a central tendency estimate. However, as shown below, in most studies and among substrates, the QQ-QQ and QQ-QR ratios provide similar results thus suggesting that either comparison may be suitable.

They represent values under consideration for intra-species TK extrapolation (i.e., UF_{AK}). For purposes of comparison, the default UF_{HK} is 3X. Thus, values which differ substantially from 3X are of particular interest. Ultimately, the UF_{AK} will be combined with the 3X UF_{HD} for the intra-species extrapolation factor.

For the majority of studies evaluated where only adults were included, the resulting ratio of 50th/5th percentile ratios were 3X or less. This suggests that for adults, the default 3X factor is a reasonable approximation of within human variability.

In the four scenarios which considered newborns and mothers, the values are substantially greater than 3X—ranging from approximately 7X up to 31X. Based on this finding, the Agency preliminarily concludes that age-related maturation is the major contributor to population variability with respect to PON1 activity. CPOase data are the most appropriate for assessing population variability with respect to detoxification of chlorpyrifos oxon. CPOase data are limited in that only one study reports CPOase data in newborns and mothers (Holland et al, 2006). For CPOase, the QQ-QQ and QQ-QR ratios provide similar results, approximately 11-12X⁵.

⁵ The Agency notes that this factor of 12X differs from the population variation estimates reported by the study authors. Holland et al, (2006) report population variation estimates of approximately 70-fold in mothers and newborns for CPOase. These values are derived from a comparison of the lowest and highest values as thus represent the minimum and maximum.

Table 9. Preliminary Results of DDEF analysis for Intra-Species Extrapolation for TK (UF_{AK}) based on PON1 Activity.

Nationality/Ethnicity	POase		ARase		CPOase	
	QQ _{50th} / QQ _{5th}	QR _{50th} / QQ _{5th}	QQ _{50th} / QQ _{5th}	QR _{50th} / QQ _{5th}	QQ _{50th} / QQ _{5th}	QR _{50th} / QQ _{5th}
American, adult female, multiple ethnic groups ⁷	1.3	3.4	Not reported		1.3	1.4
American, adult male, multiple ethnic groups ⁷	1.4	3.5	Not reported		1.3	1.2
African-American, newborns ⁵	Not reported		2.6	2.7	Not reported	
African American, mothers ⁵			1.6	1.8		
<i>African American, mothers & newborns</i> ⁵			7.2	8.3		
Caucasian; ⁴			2	2		
Caucasian newborns ⁵			2.3	2.3		
Caucasian mothers ⁵			1.5	1.4		
<i>Caucasian mothers & newborns</i> ⁵			12.0	11.4		
Hispanic newborns ⁵			2.2	2.1		
Hispanic mothers ⁵			1.7	1.7		
<i>Hispanic mothers & newborns</i> ⁵			7.8	8.2		
Iranians ²	1.7	4.6	1.3	1.1		
Latino newborns ⁶ (n = 130)	2.2	8.2	3.1	4	2.3	3
Latino mothers ⁶ (n = 130)	1.6	5.1	1.6	1.6	1.7	1.8
<i>Latino mothers & newborns</i> ⁶	9.9	31.2	18.5	17.5	10.8	11.6
Peruvians ¹	1.9	3.1	1.9	2.2		
Workers (high OP exp) ³	1.1	2.3	1.1	1.1		
Workers (low OP exp) ³	1.1	2.3	1.1	1.1		

¹ Catano, et al (2006), ² Sepahvand, et al (2007), ³ Sirivarasei, et al (2007), ⁴ Brophy, et al (2001), ⁵ Chen, et al (2003), ⁶ Holland, et al (2006), ⁷ Kisicki et al (1999)

PON1 activity is affected by many things including genetic status in the coding region such as the PON1-192 and PON1-55 genotypes, but also in the regulatory region (Brophy et al, 2001; Deakin et al, 2003). For example, Deakin et al (2003) describe three polymorphisms in the PON1 promoter; each polymorphism leads to differences in activity. Furthermore, PON1 activity can be affected by environmental factors like smoking (Nishio and Watanabe, 1997; James et al, 2000; Jarvik et al, 2000), fat content in the diet (Shih et al, 1998; Hedrick et al, 2000), consumption of antioxidants (Aviram et al, 2000) or consumption of alcoholic beverages (Hayek et al,

1997; van der Gaag et al, 1999). In pregnant women and umbilical cord blood, POase activity can also be affected by duration of labor or the type of delivery (Viachos et al, 2006). It is interesting to note that the PON1-192 R allele has been associated with pre-term births (Lawlor et al, 2006; Chen et al, 2004). With regard to changes during pregnancy, available studies show different results. Ferre et al (2006) showed a decrease in paraoxon hydroxylation of approximately 25% in late gestation compared to nonpregnant background levels. Carpintero, et al (1996), however, found that phenyl acetate metabolism increased from approximately 40% in the third trimester.

Serum A-esterase levels are very low in human infants compared to adults (Augustinsson and Barr, 1962; Mueller et al., 1983; Ecobichon and Stephens, 1973; Holland et al, 2006; Chen et al, 2003). After birth, there is a steady increase of this activity (Augustinsson and Barr, 1962). Similarly, Burlina et al (1977) evaluated age-dependence of total serum arylesterase activity and showed that adult levels were achieved by two years-of-age. The Agency is aware of yet unpublished data on POase, ARase and CPOase in children up to age 5 from Drs. Nina Holland and Brenda Eskinazi with a much larger sample size (>200) than previous studies. These data were presented at the ASHG (Huen et al, 2007) and suggest that POase activity may be lower than adult levels up to 47 months. After completion of the data analysis and ultimately publication, these data will substantially improve the overall understanding of the human ontogeny of POase, ARase and CPOase. The findings of the older literature (Augustinsson and Barr, 1962; Mueller *et al.*, 1983; Ecobichon and Stephens, 1973) combined with more recent studies by Holland et al (2006) and Chen et al (2003) support a similar conclusion that newborns and young children have lower levels of PON1 than do adults. More specifically, newborns have lower levels of PON1 than do other age groups. As such, these findings suggest that development and use of a DDEF for intra-species TK extrapolation from newborn and maternal data would be protective of other age groups since PON1 levels are expected to be lowest at birth.

Some have suggested that PON1 status is a key contributor in chlorpyrifos sensitivity whereas others have suggested that a significant amount of OP must be present in the blood or brain for PON1 activity to affect toxicity based on generally low affinity (K_m , 0.1-10 mM; Aldridge and Reiner, 1972; Fonnum and Sterri, 2006; Timchalk et al, 2002b). This concept, namely relevance of PON1 at environmentally relevant concentrations, is key for determining its potential use in human health risk assessment. In addition, a key uncertainty in the use of PON1 data in the risk assessment is the extent to which reliance on population variability from a single enzyme (i.e., PON1) reflects actual variability given that multiple detoxification pathways are functioning which may modulate deficits. The Agency has considered data from multiple sources in this evaluation: information from PBPK model simulations, *in vitro* studies, animal studies, and human epidemiology.

PBPK Model Simulations: PBPK modeling is a valuable tool as it provides a computational approach to evaluate the relative importance of specific metabolic parameters such as the relatively high K_m of PON1. Moreover, a PBPK model involves

consideration of multiple pathways simultaneously and thus can consider the extent to which one or more metabolic pathways may modulate deficits in other pathways.

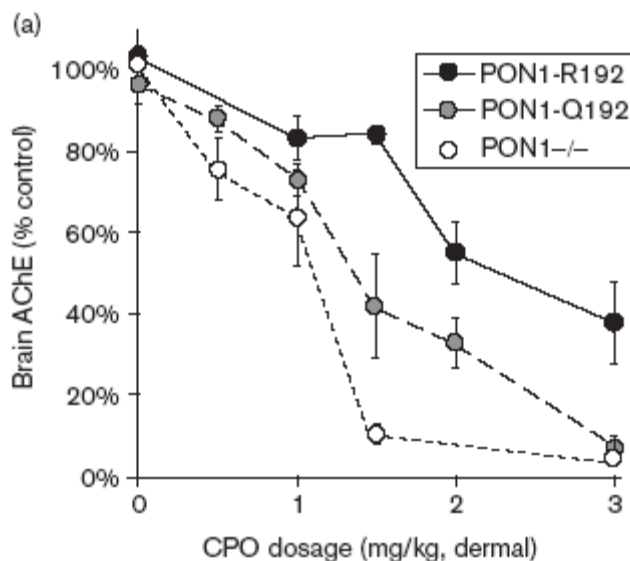
Timchalk et al (2002b) performed Monte Carlo analysis of PON1 levels from adults for the QQ, QR, and RR genotypes using the chlorpyrifos PBPK model. In these simulations, at lower doses (~5 µg/kg) CPOase was not a determinant in the outcome. However, at higher doses (~0.5-5mg/kg), the authors suggest that CPOase may be a determinant in toxicity. The authors further suggest that other esterase detoxification pathways may adequately compensate for lower CPOase activity; hence an increased sensitivity to low CPOase is not observable until other detoxification pathways or esterases have been appreciably depleted or overwhelmed.

The same group of investigators has used PBPK modeling to evaluate changes in PON1 consistent with newborn levels and changes during pregnancy (Public comment to the FIFRA SAP by Drs. Poet and Timchalk). The PBPK simulations reported in the public comment provide similar findings as Timchalk et al (2002b) in that reductions in PON1 levels, including levels consistent with the 12-fold DDEF shown in Table 3, did not have substantial impact on BuChE inhibition levels. The models discussed in the public comment have not been through substantial peer review and have not been published in the literature. Moreover, they do not provide information on dose or effects to the fetus and on effects in children younger than 5 years old. They do, however, provide information that suggests that at environmentally relevant concentrations of chlorpyrifos, PON1 status may not be a determinant in toxicity in older toddlers and adults.

In vitro data: Due to the relatively high K_m of PON1, Mortenson et al (1996) tested the ability of CPOase to hydrolyze the oxon at physiologically relevant concentrations (e.g., nM to low µM). Mortenson et al (1996) reported that CPOase activity in rats was indeed capable of hydrolyzing physiologically relevant concentrations of chlorpyrifos oxon; thereby suggesting that CPOase may hydrolyze the oxon at low environmental concentrations. In a recent study by Sogorb et al (2008), serum albuminase activity was compared to POase, CPOase, and diazoxonase. At concentrations of chlorpyrifos oxon up to 5 µM, CPOase was effective at preventing any meaningful reductions in AChE activity. On the other hand, a clear dose-dependant increase in AChE inhibition was noted for serum albuminase. As stated by the authors, “the activity associated with PON1 was able to fully protect AChE in the case of chlorpyrifos-oxon, where the contribution of albumin was barely significant.”

In vivo animal data: To investigate the role of PON1 on chlorpyrifos sensitivity, Cole et al (2005) used a transgenic mouse model which expresses human PON1Q192 or PON1R192 at equivalent levels in the absence of endogenous mouse PON1. The investigators compared effects of chlorpyrifos and the oxon following dermal exposure to mice. They showed that adult mice expressing PON1-Q192 were significantly more sensitive to the oxon than were mice expressing PON1-R192. As shown in Figure 7, this sensitivity was evident at all tested doses but was more pronounced at higher doses.

Figure 7. Dose–response of chlorpyrifos oxon on inhibition of brain AChE activity. Extracted from Figure 2.a in Cole et al (2005).



Studies in agricultural workers: There are a couple studies available which have evaluated effect of PON1 status in agricultural workers who handle OPs; these studies provide inconsistent findings. It is important to note that all three studies are limited to varying degrees and suffer from many of the same weakness as other case controlled studies. For example, each is limited by small sample size, the amount of exposure information collected, and recall bias. Both studies provide little exposure information, including which OPs that the workers were exposed to. Povey et al (2005) evaluated sheep dippers in the UK who reported chronic ill health and have handled OPs. In this study, for self-reported symptoms consistent with OP poisoning, odds ratios for QR or RR genotype were approximately 2-fold higher than those for the QQ genotype. Lee et al (2003) reported an increased incidence of reported symptoms consistent with chronic OP exposure in QQ or QR genotypes in 100 workers in South Africa (odds ratio of 2.9, confidence interval 1.7-6.9).

Epidemiology studies in children & mothers: With regard to effects in children, three publications by the same group at Mt. Sinai, New York report associations between maternal PON1 activity and birth outcomes (Engel et al, 2007; Berkowitz et al, 2004; Wolff et al, 2007). PON1 activity measurements for the Mt. Sinai cohort are found in Chen et al (2003).

In Berkowitz et al (2004), maternal levels of TCP above the limit of detection in combination with low maternal PON1 activity were associated with a significant (albeit small) reduction in head circumference. Berkowitz et al (2004) further reported that maternal PON1 levels, not PON1 genetic polymorphisms, were associated with reduced head size. In a follow up study, Engel et al (2007) reported that abnormal reflexes were associated with total dimethylphosphate (DMP) metabolites when ARase activity was included in the analysis. Diethylphosphates⁶ (chlorpyrifos is a diethylphosphate; DEP) and total di-alkylphosphate (DAPs) were associated with abnormal reflexes without inclusion of PON1 status in the analysis. Wolff et al (2007) evaluated the association between DAP levels and birth outcomes from the same cohort. With the lowest tertile ARase activity, urinary DEPs were associated with lower birth weight and DMPs with shorter birth length. Wolff et al (2007) also reported that birth length was shorter for RR mothers compared with QQ mothers.

As of August, 2008, the Agency was not aware of any studies published in the literature evaluating PON1 status and health outcome in children from the CHAMACOS cohort. PON1 status has been measured in the CHAMACOS cohort and reported by Holland et al (2006). The investigators have communicated plans to present data on PON1 status and birth outcomes at the upcoming ISEE/ISEA meeting (2008). The Columbia University investigators have not measured PON1 status in the mothers or children in the other NY cohort.

In summary, animal studies using *in vivo* studies in transgenic animals and *in vitro* techniques support that PON1 status effects sensitivity to chlorpyrifos oxon. Studies in transgenic animals must be interpreted with care as they represent an artificial model---human genes expressed in the mouse. Moreover, the Cole et al (2005) evaluates primarily high doses. Human epidemiology data on agricultural workers and in children are limited. Results of epidemiological studies in workers would be more convincing with larger sample sizes⁶ and a prospective study design. The reported associations reported by Engel et al (2007), Berkowitz et al (2004) and Wolff et al (2007) in children would be more convincing if similar findings were available in another cohort of children and mothers. Such data from the CHAMACOS cohort may be available in late 2008 or early 2009.

Key preliminary conclusions in the chlorpyrifos hazard characterization are: 1) juveniles are more sensitive than adults and 2) this sensitivity is derived, at least in part, based on TK differences in young and adults, including PON1 (or A-esterase). There are remaining uncertainties regarding the relevance of PON1 at environmentally relevant concentrations and further uncertainties regarding the extent to which other detoxification pathways modulate deficiencies in PON1 activity. However, on balance, population variability with respect to PON1 status can not be ruled out as a determinant in tissue dose, and ultimately toxicity, to the fetus or to very young children. As shown below, the Agency is proposing two options for the intra-species TK extrapolation factor (UF_{HK}). The first option involves using a UF_{HK} of 12X derived from PON1 data (from CPOase activity in newborns and mothers (Holland et al, 2006; Table 4)) which would

⁶ Chlorpyrifos is a diethylphosphate OP.

lead to an intra-species extrapolation factor of 36X when combined with the 3X for UF_{HD} . The other option involves using the default factor of 3X for both the UF_{HK} and the UF_{HD} which would lead to an intra-species factor of 10X. The Agency will solicit comment on the science which supports both of these options.

4.0 Summary & Next Steps

The Agency has performed a review of the scientific literature under the context of its use in human health risk assessment. This review provides the foundation for proposed PoDs and UFs. Both the preliminary conclusion from the literature review and the proposed PoDs and UFs will be reviewed by the SAP in September, 2008.

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Attachment 1.0. RBC and brain ChE activity in dams and fetuses from comparative ChE studies following gestational exposure (From USEPA, 2006, Section II.B.2)

OP	Cholinesterase & Group	Dose (mg/kg/day)				
		0	0.5	1	2.5	10
Acephate MRID 46151805	Dose					
	GD 21 Dams RBC	1.6360 ± 0.7461	1.9691 ± 0.7684	2.3221 ± 0.5884	1.4638 ± 0.7615	1.5202 ± 0.6202
	Brain	8.6009 ± 1.4779	7.1673 ± 0.8621 (17)	7.0441 ± 0.900 (18)	5.096 ± 0.933 (41)	3.3112 ± 0.5209 (62)
	GD 21 Fetuses RBC	1.7284± 0.5776	1.9883 ± 0.7651	1.4476 ± 0.2403	1.0662 ± 0.3121	1.3385± 0.5334
	Brain	1.4688 ± 0.0871	1.3613 ± 0.1320	1.2915 ± 0.1313 (12)	1.2586 ± 0.1666 (14)	0.8816 ± 0.1254 (40)
	Dose	0	0.2	0.9	1.2	
Azinphos methyl MRID 46291101	GD 20 Dams RBC	1.43 ± 0.31	1.41 ± 0.30 (1)	1.39 ± 0.43 (3)	1.05 ± 0.20 (27)	
	Brain	11.1 ± 0.5	10.8 ± 0.7 (3)	10.0 ± 1.9 (10)	10.7 ± 0.7 (4)	
	GD 20 Fetuses RBC	1.36 ± 0.28	1.30 ± 0.07 (4)	1.31 ± 0.15 (4)	1.32 ± 0.17 (3)	
	Brain	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	2.2 ± 0.1 (0)	

OP	Cholinesterase & Group	Dose (mg/kg/day)			
Chlorpyrifos MRID 44648102 % activity compared to control	Dose	0	0.3	1	5
	GD 20 Dams RBC		73.7**±14.5	17.6**±6.7	4.9**±2.8
	Hindbrain		101.1±7.2	92.0*±2.2	24.0**±4.8
	GD 20 Fetuses RBC		102.2±20.3	106.4±16.7	7.9**±4.3
	Hindbrain		107.0±5.0	99.7±5.6	46.1**±9.3
Diazinon MRID 45842602	Dose	0	0.084	0.825	26.23
	GD 20 Dams RBC	1.106± 0.163	1.183 ±0.165	0.719± 0.223 (35)	0.00± 0.00 (100)
	Brain	17.272± 1.041	16.925± 1.066	16.675± 0.617	3.228 ±0.229 (81.3)
	GD 20 Male fetuses RBC	1.188± 0.230	1.392 ±0.183	1.319± 0.230	0.247 ±0.162 (79.2)
	Brain	2.383 ±0.194	2.380± 0.262	2.194 ±0.161	1.689± 0.348 (29.1)
	GD 20 Female fetuses RBC	1.208 ±0.143	1.325± 0.172	1.363± 0.254	0.217± 0.148 (82.0)
	Brain	2.311± 0.198	2.360± 0.395	2.231± 0.234	1.822± 0.372 (21.2)
Dicrotophos MRID 46153201	Dose	0	0.05	0.2	1.0
	GD 20 Dams RBC	2593 ± 218	2342 ± 79 (10)	1638± 120 (37)	1282 ± 226 (51)
	Brain	4.78 ± 0.99	4.26± 1.06 (10)	2.49± 0.51 (48)	1.03 ± 0.21 (78)
	GD 20 Male fetuses RBC	2546± 112	2423± 351	1923± 190 (24)	1311± 124 (49)
	Brain	1.75± 0.34	1.51± 0.25 (14)	1.22± 0.28 (30)	0.77± 0.08 (56)
	GD 20 Female fetuses RBC	2523 ± 455	2362± 50	1825± 207 (28)	1414± 142 (44)
	Brain	1.57± 0.18	1.36± 0.13 (13)	1.22± 0.11 (24)	0.72± 0.02 (54)

OP	Cholinesterase & Group	Dose (mg/kg/day)			
		0.0	0.1	0.5	3.0
Dimethoate MRID 45529702	Dose	0.0	0.1	0.5	3.0
	GD 20 Dams RBC Brain	1669 ± 180 12,838 ± 1373	1563 ± 224 (6) 13,044 ± 530 (-2)	1459 ± 278 (13) 11,563 ± 300 (10)	709 ± 104 (58) 5094 ± 1081 (60)
	GD 20 Fetuses RBC Brain	1213 ± 79 1781 ± 175	1225 ± 98 (-1) 1569 ± 173 (12)	1181 ± 172 (3) 1600 ± 136 (10)	834 ± 183 (31) 1188 ± 164 (33)
Disulfoton MRID 46635901	Dose	0	0.042	0.168	0.694
	GD 20 Dams RBC Brain	2.02±0.34 11.97±0.53	1.66±0.31 (18) 11.35±0.50 (5)	1.13±0.37 (44) 8.12±0.44 (32)	0.20±0.13 (90) 1.76±0.19 (85)
	GD 20 Fetuses RBC Brain	1.27±0.16 1.81±0.30	1.21±0.20 1.75±0.28	1.02±0.19 (20) 1.74±0.26	0.22±0.11 (83) 1.18±0.21 (35)
Fosthiazate Not yet assigned	Dose	0	0.1	0.7	5
	GD 20 Dams RBC Brain	3931± 1474.5 49446± 2189.8	3831 ± 757.3 48974 ± 1364.5	2193 ± 712.2 (44) 47135 ± 1510 (5)	20 ± 0.0 (99) 5152± 1718.9 (90)
	GD 20 Fetuses RBC Brain	2644± 644.1 6612 ± 679.5	3283 ± 992.4 6328 ± 476.3	2893± 738.3 6251 ± 649.5 (5)	1851 ± 593.4 (30) 5182 ± 684.5 (22)
Methamidophos MRID 46660901	Dose	0	0.10	1.03	3.12
	GD 20 Dams RBC Brain	1.64 ± 0.286 10.82 ± 0.271	1.68± 0.220 10.40± 1.711	0.84± 0.117 (49) 4.86 ± 0.416 (55)	0.45 ± 0.118 (73) 2.32± 0.173 (79)

OP	Cholinesterase & Group	Dose (mg/kg/day)			
	GD 20 Fetuses RBC Brain	1.29 ± 0.196 1.56 ± 0.157	1.13 ± 0.147 1.51 ± 0.089	0.72 ± 0.133 (44) 1.08 ± 0.125 (31)	0.38 ± 0.075 (55) 0.77 ± 0.061 (51)
Methyl parathion MRID 45646501	Dose	0	0.03	0.30	0.60
	GD 20 Dams RBC Brain	1500.1 ± 255.03 13.48 ± 0.807	1702.3 ± 386.36 13.58 ± 0.428	979.5 ± 283.80 (35) 12.26 ± 0.527 (9)	632.9 ± 124.52 (58) 9.35 ± 1.026 (31)
	GD 20 Male fetuses RBC Brain	1041.3 ± 145.79 2.10 ± 0.116	1082.2 ± 160.9 2.05 ± 0.095	1075.0 ± 135.32 2.04 ± 0.173	808.9 ± 186.38 (22) 1.97 ± 0.073
	GD 20 Female fetuses RBC Brain	1090.4 ± 163.7 2.06 ± 0.152	1118.0 ± 131.13 2.12 ± 0.14	1010.2 ± 130.36 2.06 ± 0.174	894.9 ± 215.77 (18) 2.02 ± 0.092
Phorate MRID 46241402	Dose	0	0.03	0.1	0.2
	GD 20 Dams RBC Brain	35.98 ± 1.12 2.95 ± 0.54	33.92 ± 3.76 2.88 ± 0.74	30.99 ± 4.82 (14) 2.94 ± 0.70	27.64 ± 5.16 (23) 1.73 ± 0.67 (41)
	GD 20 Male fetuses RBC Brain	7.05 ± 0.83 0.57 ± 0.01	5.72 ± 0.51 (19) 0.58 ± 0.04	5.69 ± 0.66 (19) 0.56 ± 0.03	6.42 ± 0.56 0.60 ± 0.03 (6)
	GD 20 Female fetuses RBC Brain	6.80 ± 0.99 0.59 ± 0.04	5.81 ± 0.91 0.57 ± 0.04	5.48 ± 0.89 0.58 ± 0.02	6.28 ± 0.78 0.59 ± 0.02
Terbufos MRID 46240802	Dose	0	0.03	0.1	0.3/0.2
	GD 20 Dams RBC Brain	42.30 ± 5.00 3.00 ± 1.12	40.68 ± 4.00 3.00 ± 0.79	14.42 ± 4.04 (66) 1.96 ± 0.68 (35)	4.46 ± 1.64 (89) 0.69 ± 0.19 (77)
	GD 20 Male fetuses RBC Brain	5.16 ± 1.48 0.59 ± 0.11	4.63 ± 1.86 0.53 ± 0.05	2.51 ± 0.86 (51) 0.48 ± 0.04 (19)	1.62 ± 0.69 (69) 0.36 ± 0.09 (39)

OP	Cholinesterase & Group	Dose (mg/kg/day)			
	GD 20 Female fetuses RBC Brain	4.32 ± 0.85 0.53 ± 0.04	4.52 ± 0.99 0.57 ± 0.04	1.99± 1.09 (54) 0.50± 0.05	1.76 ± 0.75 (59) 0.36± 0.07 (32)