Appendix E:
Data Derived Extrapolation Factor Analysis

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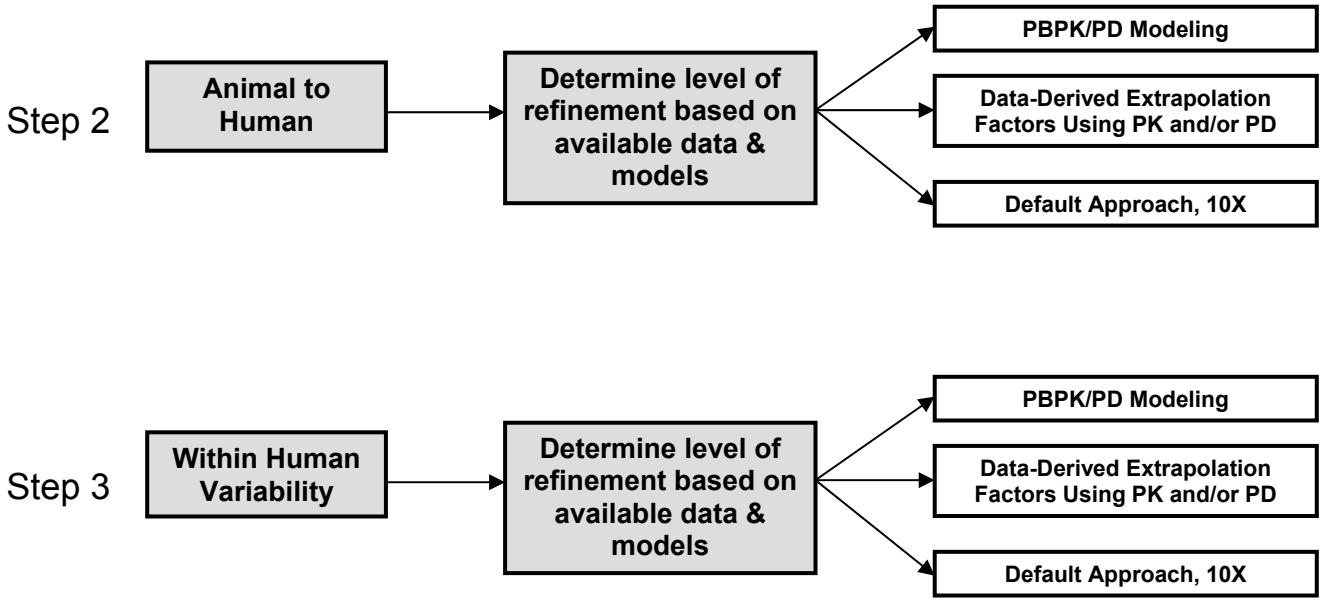
1.0 Introduction

Chlorpyrifos (0,0-diethyl-0-3,5,6-trichloro -2-pyridyl phosphorothioate) is a broad-spectrum, chlorinated organophosphate (OP) insecticide. Chlorpyrifos is one of the most widely used OPs in the U.S. In 2000, nearly all residential uses were cancelled but agricultural use remains. The Agency is in the early stages of updating and revising the chlorpyrifos human health risk assessment. As part of the re-evaluation and update of the hazard characterization and identification for chlorpyrifos, the Agency has developed an Issue Paper to summarize and interpret recent scientific literature on chlorpyrifos under the context of human health risk assessment. One aspect of the hazard assessment being considered in the updated by the Agency is the approach used in inter- and intra-species extrapolation. The Issue Paper describes three key steps in the hazard identification update for chlorpyrifos. This Appendix focuses on Step 2 (inter-species extrapolation) and Step 3 (intra-species extrapolation). The Issue Paper describes various options for Step 1 (selection of a point of departure).

Figure 1. Schematic of Steps & Decision Points in the Updated Chlorpyrifos Hazard Assessment.



Historically in oral and dermal risk assessment, EPA has used default 10X factors for extrapolating from animals to humans and within human variability. In previous risk assessments, the Agency has applied these default 10X factors for both inter- and intra-species extrapolation in addition to the FQPA 10X for chlorpyrifos. The preferred approach to inter- and intra-species extrapolation would be the use of a sophisticated model like physiologically-based pharmacokinetic (PBPK) and/or biologically based dose response (BBDR) model. PBPK models have been published for chlorpyrifos (Timchalk et al, 2002, 2007; Rigas et al, 2001; Knaak, et al, 2004; Georgopoulos et al, 2008).

The model(s) developed by Dr. Charles Timchalk and co-workers at of Pacific Northwest National Laboratory has been the most extensively developed. The Timchalk model was first published in 2002 as an adult rat and human model (Timchalk et al., 2002a) and has been updated as more data has become available (Poet et al. 2003; Poet et al. 2004; Slikker et al. 2005; Timchalk et al. 2002b; Timchalk et al. 2003; Timchalk et al. 2005). More recently, Timchalk et al. (2007) published a similar model for juvenile rats. The “scaled up” version (i.e., scaled from rats to humans) of the juvenile rat model has not been published. The unpublished version (as in the Public Comment submitted by Drs. Torka Poet and Charles Timchalk) evaluates children who are approximately 5 years old. This model for children can not assess effects in children in young toddlers and newborns. The PBPK models also do not include a placental compartment and thus can not estimate in utero exposure to the fetus. Thus, the current PBPK models for chlorpyrifos are not able to quantitatively evaluate key sub-populations, pregnant women, fetuses, and young children.

The Agency does, however, believe that the Timchalk PBPK model provides a valuable tool which can be used to assess variability in metabolic parameters. By changing the value of specific parameters, the general direction and overall magnitude of change with regard to urinary output, blood levels of chlorpyrifos or TCP, and/or butyrylcholinesterase (BuChE) inhibition can be observed. At the request of Agency, the Drs. Charles Timchalk and Torka Poet performed a series of simulations adjusting parameters to mimic the characteristics of a pregnant woman and a 5 year old child. These simulations have been provided in a Public Comment to the FIFRA SAP docket.

In 2005, the WHO published its guidance for developing Chemical Specific Adjustment Factors (CSAFs, WHO, 2005). This guidance is based in large part on analyses by Renwick (1993) and Renwick and Lazarus (1998) and describes the use of toxicokinetic (TK) and toxicodynamic (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. Typically, EPA uses the terms “Data-Derived Extrapolation factors or DDEFs” to describe extrapolation factors based on TK or TD data for a particular chemical. As such, this appendix uses “DDEF” instead of “CSAF.”

2.0 Background

When taking a data-derived approach to developing extrapolation factors, the uncertainty factor (UF) for inter-species extrapolation (UF_A) and the UF for intra-species extrapolation (UF_H) are separated into two components---TK and TD. TK and/or TD information from *in vivo* or *in vitro* studies can be used to inform a data-derived factor instead of the application of a default value. These TK or TD data can be specific to the chemical of interest, apply to a class of chemicals, or relate to chemicals sharing a mode of action.

Data on the quantitative differences in the TK between animals and humans are used for interspecies extrapolation (UF_{AK}); differences in susceptibility within the human population are used for the intra-species extrapolation (UF_{HK}). Thus the factor UF_{AK} accounts for extrapolation from laboratory animals to the general human population. The UF_{HK} factor accounts for the variation between the general human and susceptible individuals or groups. Similarly data on the quantitative differences in the TD between animals and humans are used for interspecies extrapolation (UF_{AD}); TD differences in susceptibility within the human population are used for the intra-species extrapolation (UF_{HD}). Thus the factor UF_{AD} accounts for extrapolation from laboratory animals to the general human population. The UF_{HD} factor accounts for the variation between the general human and susceptible individuals or groups.

Data may be available to develop a DDEF for one or more components of extrapolation (e.g., data for UF_{HK} but not UF_{HD}). DDEFs and defaults can be used in combination. In the IPSC CSAF guidance, the inter-species factor is separated into default values of 4.0X and 2.5X for TK and TD, respectively, while the intra-species factor is separated into 3.2X and 3.2X for TK and TD. When using DDEFs, typical practice within EPA for oral studies has been to separate the 10X default into 3X and 3X for both TK and TD. The composite factor is calculated after the appropriate DDEFs for inter- and/or intra-species differences in TK and TD. The composite factor is calculated by multiplying the specific UFs (default and/or data-derived values), as shown below. This is entirely analogous to calculating composite UF when using the 10X defaults for UF_A and UF_H. The composite factor may be less or greater than 100.

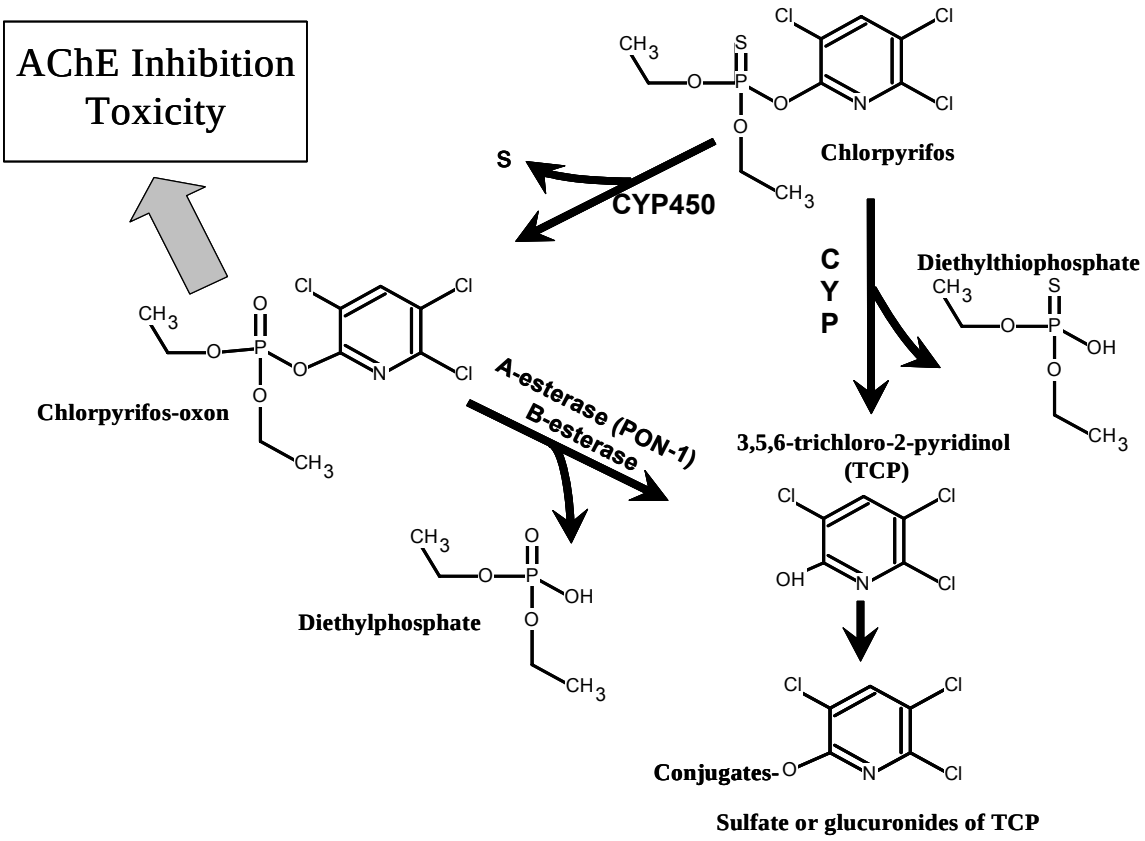
$$CF = UF_{AK} \times UF_{AD} \times UF_{HK} \times UF_{HD}$$

where:
CF = composite uncertainty factor,
UF_{AK} = uncertainty factor for interspecies extrapolation covering toxicokinetics
UF_{AD} = uncertainty factor for interspecies extrapolation covering toxicodynamics
UF_{HK} = uncertainty factor for intra-species extrapolation covering toxicokinetics
UF_{HD} = uncertainty factor for intra-species extrapolation covering toxicodynamics

The Agency has evaluated the extent to which data are available on chlorpyrifos to apply DDEFs instead of defaults. As described in detail in Appendix A, the metabolic pathway for chlorpyrifos has been elucidated in rats and humans and the two species generally show good concordance (Figure 2). There are multiple pathways by which

chlorpyrifos is detoxified and/or activated. These include carboxylesterases, butryl cholinesterase (BuChE), A-esterase such as paraoxonase1 (PON1), and P450s (which can activate or detoxify). As discussed in the Issue Paper, the focus of the Agency's current effort is to evaluate effects of chlorpyrifos to juveniles from in utero or post-natal exposure. Age-dependant sensitivity has been shown to be associated to a large degree with the maturation of detoxification enzymes. The Agency's preliminary DDEF analysis has been developed under this context (i.e., focused on studies which inform animal to human extrapolation and within human variability for gestational or post-natal TK or TD characteristics).

Figure 2. Major metabolic pathways of chlorpyrifos metabolism (Reproduced from Timchalk et al 2006).



3.0 Toxicodynamics (TD, UF_{AD} and UF_{AD})

Understanding mode of action (MOA) is an important component of deriving DDEFs in that MOA provides the foundation for understanding which TD factors are critical for extrapolation. As described in detail in the Issue Paper and Appendix C, chlorpyrifos may have multiple modes and/or mechanisms of action. When developing

data-derived factors, it is necessary to consider each mode of toxicity, critical effect, and/or target organ because the magnitude of extrapolation factors may differ among different toxicities.

The mode of action 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 a UF_{AD} and possibly UF_{HD} since 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 (See Appendix C). Given the remaining uncertainty regarding the mechanisms(s) of action affecting the developing brain, the Agency has elected not to develop a DDEF for UF_{AD} or UF_{HD}. As such, the Agency will apply the default 3X for inter- and intra-species TD extrapolation (i.e., UF_{AD} and UF_{HD}).

4.0 Toxicokinetics (TK, UF_{AK} and UF_{AK})

TK is concerned with delivery of a biologically active chemical species to the target tissue of interest. Rat fetuses and juveniles and newborn humans have lower capacities to detoxify than adults. This decreased capacity to detoxify has been associated with increased sensitivity in rats to chlorpyrifos and its oxon. Consistent with the focus in the Issue Paper on pregnant women and juveniles, age-dependant maturation of detoxification enzymes is an important consideration in this analysis. For inter-species extrapolation, an important consideration is the appropriate matching of developmental stage between rat and human. As noted by Ginsberg et al (2004b), many enzyme systems mature rapidly in juvenile animals, particularly in the first 2-3 weeks of life in rat and in the 2-3 months in a human. Due to these different time scales, direct extrapolation from rat to human using juvenile data is problematic and uncertain (Ginsberg et al, 2004b). For intra-species extrapolation, data evaluating both juveniles and adults are preferred. As such, data only performed in non-pregnant adult animals and/or adult humans provide little to no information on age-dependant sensitivity. The following text on TK is separated into two sections: 1) evaluation of available data (4.1) and 2) DDEF analysis (4.2).

4.1. Key Metabolic Enzymes

4.1.1. Carboxylesterases

As discussed in Appendix A, carboxylesterases act to detoxify chlorpyrifos by binding the oxon. There are a few studies which evaluated carboxylesterases in adult human tissues (Jewell et al, 2007; Buratti and Testai, 2005; Hosokawa et al, 1995). These studies suggest that among individuals carboxylesterase activity varies by different substrates. Given that none of these studies evaluated carboxylesterase

activity on chlorpyrifos there would be uncertainty associated with use of data from other compounds for the chlorpyrifos risk assessment.

The expression of carboxylesterases has been highly correlated with OP sensitivity during both maturation and aging (Benke and Murphy, 1975; Karanth and Pope, 2000; Moser et al., 1998). There are, however, 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). Although the Pope et al (2005) study fills an important gap in overall understanding, this study is limited for use in DDEF development by the number of samples (n=10). It is notable: however, that 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. Based on the graphs provided in Pope et al (2005), the 2-month individual had the lowest level of carboxylesterase.

Based on the limited amount of information specific to chlorpyrifos and/or investigating carboxylesterase activity in juveniles, the Agency has elected not to pursue carboxylesterase in deriving DDEFs for inter- or intra-species extrapolation.

4.1.2. Butryl Cholinesterases (BuChE)

With regard to plasma BuChE 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 close to pre-pregnancy levels in the third trimester. Similarly, Venkataraman et al (1990), Whittaker, et al (1988), and De Peyster et al (1994) 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.

Regarding exposure to juveniles, specifically the ontogeny of AChE, Ecobichan and Stephens (1973) showed a steady increase in AChE and ChE levels of infants beginning at birth up to adult levels. Actual data were not provided in Ecobichan and Stephens (1973); only plots are provided. Genc et al (1997) evaluated ChE activity in ages 0 to 93 and reported only small differences among the groups and importantly for this effort did not separate data from ages 0-5 years old. There are several studies which have evaluated post-natal increases in rat fetal brain or heart ChE activity level as the pup ages (Lassiter et al, 1998a ; Nyquist-Battle, 1990; and Slavikova and Tucek, 1986). Based on the limited amount of information in juveniles, the Agency has elected not to pursue BuChEs in deriving DDEFs for inter- or intra-species extrapolation.

4.1.3. P450's

There is a wealth of information on human and rat cytochrome P450's in the literature, including from newborns and children. This literature has been reviewed recently by several researchers (Ginsberg et al, 2002, 2004a, 2004b; Hattis et al, 2003; USEPA, 2001). In addition, much of the cytochrome P450 data available have been compiled (Dome et al, 2001; Ginsberg et al, 2002; Hattis et al, 2003; Renwick et al, 2000).

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; Vieria et al, 1996; Tateishi et al, 1997). Less is known about the development of CYP2B6 as this one is not as extensively studied as other CYPs.

The Agency has not developed a detailed DDEF analysis for the CYPs either for inter- or intra-species extrapolation. With regard to the inter-species extrapolation, quantitative comparison of juvenile rats with newborns and children is problematic. The timing of maturation of enzymes differs between rats and humans which makes appropriate matching across age challenging. Regarding intra-species extrapolation, Hattis et al (2003) evaluated ratios of child/adult elimination ½-lives for a variety of pathways and enzymes using data from the pharmaceutical literature; two of these included CYP1A2 and CYP3A. Hattis et al (2003) showed that the typical 3-fold intra-species TK factor does not account for all the individuals in their analysis. The Agency notes that Ginsberg et al (2004a) reviewed the cytochrome P450 literature for its utility in risk assessment and concluded that *"At present, it is difficult to draw quantitative inferences (e.g., child-specific TK adjustment factors)…because the way in which factors interact to modulate internal dose needs to be tested in children's PBTK analyses."* The Agency preliminarily has come to a similar conclusion.

4.1.4. A-esterase, Paraoxon-1 (PON1)

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). PON1 is also associated with high density lipoprotein particles. The role of PON1 as a potential factor in cardiovascular and other diseases is beyond the scope of this issue paper. For readers interested in this area of research, several reviews are available (Durrington, et al, 2001; Mackness et al, 2002; Costa et al, 2003; Draganov and LaDu, 2004).

With regard to inter-species extrapolation for the chlorpyrifos risk assessment, there are data available in PND4 pups (Mortenson et al, 1996) and in newborn humans (Holland et al, 2006; Chen et al, 2003). The Agency considered these studies in the DDEF analysis for inter-species extrapolation. Based on communication with Dr. Nina

Holland, the laboratory assay methods used in the rat PND4 data (Mortenson et al, 1996) are sufficiently different from those used to assess human newborns (Holland et al, 2006; Chen et al, 2003) to preclude their use in DDEF development. As such, these studies have not been used to inform the inter-species TK extrapolation factor (i.e., UF_{AK}).

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). In pregnant women and umbilical cord blood, POase activity can also be affected by duration of labor or the type of delivery (Vlachos et al, 2006). 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 (1997), however, found that phenyl acetate metabolism increased from approximately 40% in the third trimester.

Regarding TK intra-species extrapolation, PON1 has been extensively studied in many populations around the world, people of different health status, and in different age groups. Population variability data this extensive are rare. The Agency has performed a series of DDEF calculations on PON1 data reported in the literature.

4.1.5. Preliminary Conclusions on UF_{AK}, and UF_{HK}

As discussed above, due, in part, to uncertainties associated with matching rat and human metabolic parameters for juveniles and, in part, to limited data in pregnant women, human babies, and children, the Agency has elected not to develop a DDEF for inter-species TK extrapolation (i.e., UF_{AK}). As such, the Agency will apply the 3X default factor for UF_{AK}. The Agency has, however, performed a DDEF analysis on PON1 data reported in the literature for purposes of evaluating intra-species extrapolation (UF_{HK}). This analysis is considered *preliminary* and represents the first attempt by the Office of Pesticide Programs (OPP) to develop an intra-species extrapolation factor based on activity of a detoxification enzyme. As such, the Agency will solicit comment from the Panel on several issues relating to the scope of the analysis, mathematical approach, and relevance of PON1 status at environmentally relevant levels.

4.2. Intra-species Extrapolation (UF_{HK}): PON1

4.2.1. Scope

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 two polymorphisms in the coding region, PON1-192 and PON1-55 genotypes, are the most reported in the literature. The L/M polymorphism at position 55 results from a Leu/Met substitution and has been associated with plasma PON1 protein levels but does not affect catalytic efficiency. 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 to chlorpyrifos oxon. Transgenic mice which express human Q192 alloform have been shown to be more sensitive to chlorpyrifos oxon than those expressing the R192 alloform.

In the preliminary analysis, the Agency has focused on the PON1-192 polymorphism since it has been studied more extensively than any other and 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.

4.2.2. Approach

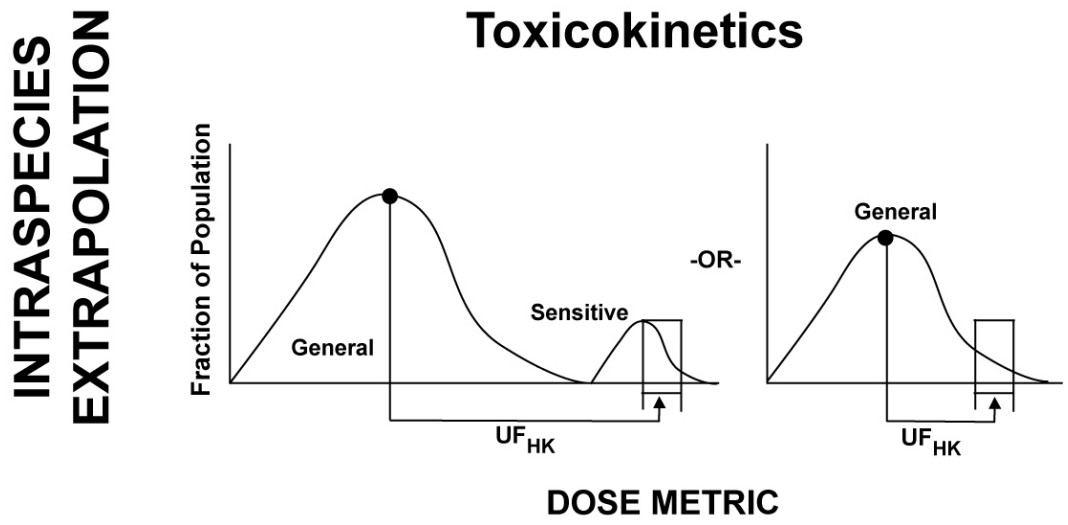
This analysis began with a survey of literature studies reporting PON1 data from different populations and genotypes. The table contained in Attachment 1 provides a list of the studies evaluated in the survey and provides information on proportions of different groups who are QQ, QR, and RR for PON1-192. These studies include data from many countries worldwide and involve many different ethnic groups. For example, Chen et al (2003) reports the highest proportion of QQ (64% in Caucasian newborns) while Scacchi et al (2003) reports fewest (6% QQ for Cayapa Indians from Ecuador). Gamboa et al (2006) reported the highest proportion of QR individuals, 70.8%, in some Mexicans.

As described in detail by Furlong et al (2005), using genetic variability data (as that shown in Attachment 1) without consideration for plasma enzyme levels and activity is of limited value since PON1 levels play an important role in the degree of metabolism. As such, the Agency collected information on paraoxonase (POase), CPOase, and arylesterase (ARase) from a subset of studies listed in Attachment 1. These represent activity on paraoxon, chlorpyrifos oxon, and aryl acetate, respectively. ARase activity has been correlated with levels of PON1 (Holland et al, 2006). Many of the studies shown in Attachment 1 only report proportions of a particular group who are QQ, QR, or RR and do not provide esterase activity and thus were not evaluated further. The Agency considered all studies which included both adults and newborns and all studies which evaluated CPOase. Sirivarasei et al (2007) was evaluated as this study included data from workers who are exposed to OPs and thus represent a potentially highly exposed population.

In this analysis, the Agency has basically followed the 2005 CSAF IPSC guidance with modifications for consistency with typical EPA practice. Conceptually, susceptibility associated with TK characteristics is due to higher target tissue concentrations of the toxicant (Figure 3). There are two potential approaches for comparing TK data from a sensitive subpopulation to the general population. In some cases, separate datasets will be available for the sensitive subpopulation and the general population (left panel). In these cases, UF_{HK} would be determined as the ratio of the dose metric at a lower percentile (e.g., 10^{th}, 5th, 2.5th, 1^{st} percentile of the distribution) for those deemed sensitive and a central tendency measure of the general population. In other cases, sensitivity may be distributed throughout the general population, and sensitive individuals may be those in the tail of the distribution (i.e., right panel, Figure 4). In this case, UF_{HK} should be determined as the ratio of the level at a lower percentile for those considered sensitive (i.e., 5th, 2.5th, 1^{st} percentile) and the level of the dose metric at a central tendency measure of the general population. Specific to PON1, typically data on genotypes and/or different age groups are reported separately. As such, the analysis described here follows the example shown in the left panel on Figure 3.

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). 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 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.

Figure 3. Intra-species (Figure provided by Dr. John Lipscomb, EPA-ORD-NCEA; from the Internal Draft of EPA's DDEF Guidance).



The IPCS CSAF guidance on animal to human extrapolations for TK advises against the use of data derived in vitro without proper physiological constraints or bounds. That guidance indicates that measures of clearance derived in vivo or in vitro, may be acceptable metrics upon which to base a non-default (chemical-specific) "adjustment" factor. One measure of clearance is intrinsic clearance, and it is expressed as the volume of fluid cleared of a substance per unit time. Intrinsic clearance is derived from metabolic rate constants estimated through studies conducted in vitro. The metabolic rate constants for saturable metabolic processes are Vmax (the theoretical maximal initial velocity of the reaction – under conditions where substrate concentration is not limiting) and Km (the substrate concentration that drives metabolic rate at one-half the maximal velocity). Intrinsic clearance is estimated as Vmax/Km.

POase, CPOase, and ARase measures reported in the literature are believed to be Vmax estimates. Available evidence suggests that it is Vmax, and not Km, that varies according to age. Ecobichon and Stephens (1973) showed that Km's for esterase activity on phenyl acetate were similar for premature and full term babies (34 and 40 weeks, respectively) and adults. Similar data in rats from Mortenson et al. (1996) have demonstrated that Km values are similar between PND4 rats and adult rats. Data that describe the similarity of Km values among different PON alleles in humans have not been located but are assumed to be similar. In the absence of data to the contrary, it seems reasonable to assume that Km is the same across alleles (QQ, QR and RR). Thus, quantifying TK differences by relying on Vmax values, rather than on estimates of intrinsic clearance (Vmax/Km) would result in the same measure of difference. Since chlorpyrifos oxonase activities were measured at single concentrations in the clinical measurements, and since it is apparent that these concentrations would result in saturating or in near-saturated metabolic rates (which may differ little from maximal rates of metabolism), it seems reasonable to quantify differences for the purposes of uncertainty factor derivation based on these clinically-measured (e.g., estimated maximal metabolic rate) differences.

The following calculations were performed.

1. Metabolic activity data from selected studies given as arithmetic means and standard deviations, and is assumed to follow a lognormal distribution (i.e., right-skewed).
2. Calculate the 5^{th} and 50^{th} percentiles using the following formulas:

$$CV = \frac{\beta}{\alpha}$$

$$\mu_g = \frac{\alpha}{\sqrt{(1 + CV^2)}} \quad = 50^{th} \text{ %tile}$$

$$\sigma_g = e^{\sqrt{\ln (1 + CV^2)}}$$

$$5^{th} \text{ %tile} = \mu_g \sigma_g^{-1.645}$$

3. Calculate $50^{th}/5^{th}$ percentile ratio for select populations:

$$\frac{50^{th}\ \%tile}{5^{th}\ \%tile}$$

CV = coefficient of variation
β = standard arithmetic deviation
α = arithmetic mean
μ_g = geometric mean
σ_g = standard geometric deviation

The Agency has provided the spreadsheet used in the calculations the Panel and public. Tables 1 and 2 provide an example calculation:

Given POase metabolic activity for various genotypes:

Table 1. Example calculations: POase metabolic activity (U/L) from Holland et al (2006)

Population	Genotype					
	QQ		QR		RR	
	Mean	SD	Mean	SD	Mean	SD
Maternal Latinos	340.7	103.7	1064	293.5	1927.9	486.5
Neonate Latinos	81.2	41.4	292.3	121.2	543.3	260.5

Calculate statistics from formulas above for desired genotypes and populations. Shown below are $50^{th}/5^{th}$ percentile ratios between and within Latino mothers and children with genotype QQ.

Table 2. Example calculations: Calculate statistics for 50th/5th percentile ratios between and within Latino mothers and children with genotype QQ from Holland et al (2006).

Population	QQ Genotype								
	Mean	SD	CV	μ_g	σ_g	50^{th}	5^{th}	$50^{th}/5^{th}$ Ratio	
Maternal Latinos	340.7	103.7	0.30	325.9	1.35	325.9	199.7	1.6	9.9
Neonate Latinos	81.2	41.4	0.51	72.4	1.62	72.4	32.8	2.2	

4.2.3. Preliminary Results

The results summarized in Table 3 are *preliminary*. The Agency will be soliciting comment from the SAP on the current analysis. The values reported in Table 4 represent values under consideration for intra-species TK extrapolation (i.e., UF_{HK}). For purposes of comparison, the default UF_{HK} is 3X. Thus, values which differ substantially (higher or lower) from 3X are of particular interest. Ultimately, the UF_{HK} 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 $50^{th}/5^{th}$ 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 11-12X[1].

[1] 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 3. 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		
African American, mothers & newborns[5]			*7.2*	*8.3*		
Caucasian;[4]			2	2		
Caucasian newborns[5]			2.3	2.3		
Caucasian mothers[5]			1.5	1.4		
Caucasian mothers & newborns[5]			*12.0*	*11.4*		
Hispanic newborns [5]			2.2	2.1		
Hispanic mothers[5]			1.7	1.7		
Hispanic mothers & newborns[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
Latino mothers & newborns[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)

4.3. Discussion

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, 1996; 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 (Vlachos 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 (1997), 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, 1963; Mueller et al*.,* 1983; Ecobichon and Stephens, 1972; Holland et al, 2006; Chen et al, 2003). After birth, there is a steady increase of this activity (Augustinsson and Barr, 1963). Similarly, Burlina et al (1977) evaluated age-dependence of total serum ARase 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 Eskanazi 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, 1963; Mueller et al., 1983; Ecobichon and Stephens, 1972) 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 (Km, 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 valuable tool as it provides a computational approach to evaluate the relative importance of specific metabolic parameters such as the relatively high Km 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.

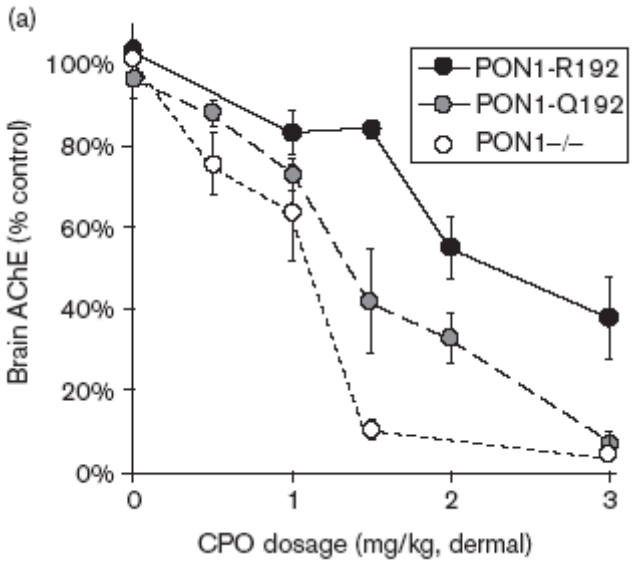
In vitro data: As discussed in the Issue Paper and Appendix A, the liver is the major site of metabolism for chlorpyrifos. The majority of population data on PON1 comes from blood, not liver. The Agency is aware of at least one study (Mutch et al, 2007) which report liver diazinonase, CPOase, and POase from 27 adult livers. Based on the results of Mortensen et al (1996), there is some uncertainty associated with using blood PON1 data, like that reported in Holland et al (2006) and Chen et al (2003), for informing the intra-species factor. However, the specific activity of plasma from PND4 rats was about 10% of the liver while in adults, specific activity in plasma was 68% of the liver activity.

In the same study, due to the relatively high Km 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 4. 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 . It is important to note that all three studies are limited to varying degrees. For example, each is limited by small sample size 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. Diethylphosphates[2] (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 sizes 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.

As discussed in the issue paper, 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 environmental 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}): one option involves using a UF_{HK} derived from PON1 data and another involves using the default factor of 3X. The Agency will solicit comment on the science which supports each of these options. Specifically, the Agency is considering applying a UF_{HK} factor of 12X derived from CPOase activity in

[2] Chlorpyrifos is a diethylphosphate OP.

newborns and mothers (Holland et al, 2006; Table 4). When combining the UF_{HK} with the 3X for UF_{HD} this would lead to intra-species extrapolation factors of 36X and 10X, respectively.

5.0 Preliminary Conclusions and Possible Calculations for the Composite Factor

The 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 mode(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 will apply the default 3X for inter- and intra-species TD extrapolation (i.e., UF_{AD} and UF_{HD}). Furthermore, 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 will apply the default 3X for UF_{AK}. As discussed in detail above, the Agency is proposing two options for the intra-species TK extrapolation factor (UF_{HK}): one option involves using UF_{HK} derived from PON1 data and another involves using the default of 3X. One possible UF_{HK} factor is 12X, derived from CPOase activity from Holland et al (2006; Table 4).

The equation below provides the calculations for combining factors for inter- and intra-species extrapolation for TD and TK and for deriving the composite factor. This composite factor can be made up of a combination of defaults and data-derived values. Potential composite factors are shown in Table 7.

$$CF = UF_{AK} \times UF_{AD} \times UF_{HK} \times UF_{HD}$$

where:
CF = composite uncertainty factor,
UF_{AK} = uncertainty factor for interspecies extrapolation covering toxicokinetics
UF_{AD} = uncertainty factor for interspecies extrapolation covering toxicodynamics
UF_{HK} = uncertainty factor for intra-species extrapolation covering toxicokinetics
UF_{HD} = uncertainty factor for intra-species extrapolation covering toxicodynamics

Table 4. Possible 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

6.0 References

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Appendix 1. Population Variability, PON1-192 Q/R Genotypes

The table below provides a survey of many studies in the literature which have reported population variability for the PON1-192 genotype. These studies include data from many countries worldwide and involve many different ethnic groups. As shown in Table 1, genotype for the 192 polymorphisms varies greatly among populations. Based on this table, for example Chen et al (2003) reports 64% QQ in Caucasion newborns while Scacchi et al (2003) reports 6% for Cayapa Indians from Ecuador. Gamboa et al (2006) observed the highest proportion of QR individuals, 70.8%, in some Mexicans.

Survey of Studies Evaluating Population Variability (% of total population) With Regard to the PON1-192 Q/R Genotype.

Citation	Nationality/Ethnicity	N	QQ	QR	RR
Populations studied in the United States					
Chen et al 2003	African-American, mothers	112	14	44	42
Chen et al 2003	African-American, newborns	51	8	43	49
Chen et al 2003	Caucasian, mothers	78	55	34.6	10
Chen et al 2003	Caucasian, newborns	47	64	30	6
Brophy et al, 2001	Caucasian, male	376	51.8	42.0	6.1
Chen et al 2003	Hispanic, mothers	200	28.5	50.5	21
Chen et al 2003	Hispanic, newborns	91	25	52	22
Holland et al 2006	Latina, mothers	130	30	46.9	23.1
Holland et al 2006	Latina, newborns	130	20	55.1	23.9
Populations studied Around the World					
Allebrandt et al 2002	Afro-Brazilian	70	21.4	51.4	27.1
Scacchi et al 2003	Bariba	49	24	36.7	38.8
Scacchi et al 2003	Berba	49	12	44.8	42.8
Mackness et al, 2001	British	282	55.3	35.1	8.5
Acuna et al 2004	Chileans (Amerindian 34.5%)	195	30	53	16
Acuna et al 2004	Chileans (Amerindian 15.9%)	129	40	52	8
Wang et al 2003	Chinese	475	10.9	48.4	40.6

Citation	Nationality/Ethnicity	N	QQ	QR	RR
Sanghera et al 1997	Chinese, Singapore	244	32.8	50	16.8
Sen-Janerjee et al 2000	Costa Rican	518	53.8	43.6	2.5
Heijmans et al 2000	Dutch	614	47.7	43.8	8.46
Leus et al, 2000	Dutch	187	48.1	42.2	9.6
Scacchi et al 2003	Ecuador, Cayapa Indians	83	6	30	63.8
Scacchi et al 2003	Ethiopian, Amhara	88	32	53	14.8
Scacchi et al 2003	Ethiopian, Oromo	81	42	36	22
Allebrandt et al 2002	Euro-Brazilian	101	48.5	41.6	9.9
Antikainen et al 1996	Finnish	169	51	44	4
Ruiz et al 1995	French	434	56	41	3
Hermann et al 1996	French, Eastern	186	49.5	39.8	10.8
Hermann et al 1996	French, Northern	144	49.3	42.4	8.3
Hermann et al 1996	French, Southwestern	201	54.2	33.8	11.9
Gardemann et al 2000	German	2784	51.0	41.5	7.47
Pati and Pati, 1998	Indians	80	75	15	10
Sanghera et al 1997	Indians, Asian	165	47	40	13
Sepahvand et al 2007	Iranian	132	48.0	42.3	9.6
Hasselwander et al, 1999	Irish	491	46	46	8
Scacchi et al 2003	Italian, Northern	179	49.7	38	12
Scacchi et al 2003	Italian, Sardinian	161	57	36	6.8
Suehiro et al 2000	Japanese	132	10.6	59.0	30.3
Imai et al, 2000*	Japanese	431	44	42	14

Citation	Nationality/Ethnicity	N	QQ	QR	RR
Rojas-Garcia et al 2005	Mexican	214	29	44	27
Gamboa et al 2006	Mexican, Amerindian	168	22.1	70.5	7.2
Gamboa et al 2006	Mexican, Mestizos	182	23.6	57.1	19.2
Hermann et al 1996	Northern Irish	170	52.9	36.4	10.7
Catano et al 2006	Peruvian	89	23.6	60.7	15.7
Hernandez et al 2003	Spanish	141	48.9	42.6	8.5
Phuntuwate et al 2005	Thai	202	50	42.1	7.9
Sirivarasai, et al 2007	Thai	90	16.6	45.6	37.8
Aynacioglu et al (1999)	Turkish	381	49.1	40.2	10.8

*Assumed reversal of RR and QQ subjects reported in paper