

**Background Information & Proposed Approach
for Using Several Chlorpyrifos Human Studies**

Background:

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 uses remain. EPA is in the early stages of developing a new risk assessment for chlorpyrifos to support registration review (http://www.epa.gov/oppsrrd1/registration_review). In addition, the National Resources Defense Council (NRDC) and the Pesticide Action Network North America (PANNA) have petitioned the Agency to cancel all remaining uses of chlorpyrifos based on a variety of science issues. 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 its mechanism of action. The Agency's forthcoming risk assessment is expected to emphasize this recent science.

In 2008 EPA brought a draft issue paper to the FIFRA Scientific Advisory Panel (SAP) for a consultation on a number of challenging science issues (USEPA, 2008). These issues included evaluation of acetylcholinesterase (AChE) data in pregnant rats, fetuses, and post-natal pups; data on behavioral effects and non-cholinergic toxicities resulting from chlorpyrifos exposure; epidemiological studies in mothers and children; and proposed points of departure (PoD) for the oral, dermal, and inhalation routes. The Panel released its report in December, 2008. Overall, the Panel responded favorably to the Agency's analyses. Although the Panel suggested additional analyses, it was in general agreement with most of the Agency's preliminary conclusions (FIFRA SAP, 2008).

In the draft issue paper, the Agency included an evaluation of the utility of the available deliberate dosing human studies. The Agency preliminarily concluded that the blood and urine measurements of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP, a metabolite used as a biomarker for chlorpyrifos) were useful in understanding and characterizing epidemiology studies but that these studies were not appropriate for deriving a point of departure (PoD) or for directly deriving an inter-species uncertainty factor. This determination was based on a variety of factors including the study design of the human studies and in particular the outcomes measured in these studies. Specifically, there are experimental laboratory animal data that indicate that the susceptibility of the developing nervous system to chlorpyrifos may be related to cholinergic and noncholinergic mechanisms. Findings in epidemiology studies in children support these animal studies. The deliberate dosing human studies do not consider toxicity endpoints other than AChE inhibition (and related clinical signs), and are therefore not useful in the forthcoming risk assessment which will focus on pregnant women and children and effects such as those on the developing brain,.

As part of the charge questions for the SAP, the Agency requested that the Panel

“comment on the clarity and completeness of the Agency's scientific analysis of the human studies. In particular, please focus on whether the Agency has identified the key scientific

issues and whether other information or studies are available that should be considered in formulating the Agency's preliminary conclusion to use these studies for purposes of characterizing and interpreting the epidemiology and biomonitoring data and not for deriving PoDs or UFs."

The Panel responded to this charge in pertinent part:

"The Panel agreed with the Agency['s] scientific analysis that these deliberate dosing studies cannot be used to directly establish a PoD or UFs. The Panel indicated that these data might be used as bounding levels similar to what was suggested by Panel concerning the data from the epidemiological studies (see Panel response to Question 5(a))."

The Panel appreciated the Agency's scientific analysis to compare the blood levels in the deliberate dosing and epidemiological studies, and considered it critically important to maximally use the information from these studies. These studies could be used for purposes of interpreting (at least crudely) the nature of the dose-response in the epidemiological data and as a basis to "bound" the reference doses/concentrations. The Panel encouraged the Agency to consider the use of a PBPK model to widen the application of these bounding data for current or potential human exposures and for the final reference dose or reference concentrations. In addition, these human study data may contribute to an array of the dose-response data in both animals and humans mentioned under question 5a, but in the context of their relative associated uncertainties (pp. 50, FIFRA SAP, 2008)."

The SAP has encouraged the Agency to make use of these data for two key purposes: 1) bounding estimations in deriving the reference dose or reference concentration and in characterizing the biomarker and exposure data from the epidemiological studies; and 2) developing a physiologically-based pharmacokinetic model (PBPK) which could aid in characterizing levels of chlorpyrifos and/or TCP based on current exposure levels.

The Agency is now seeking review by the HSRB of four human studies conducted with chlorpyrifos. The Agency acknowledges that the proposed use of the chlorpyrifos human data differs sharply from the use made of human data on previous chemicals brought before the Board. In previous cases the Agency has proposed either to use a study as a PoD or to use a study to directly inform the inter-species uncertainty factor. In this case, however, the Agency believes the blood and urine data collected from these deliberate dosing studies will help the Agency to better characterize the outcomes reported in epidemiology studies in mothers and children, to link those outcomes with the results from animal studies, and ultimately to more accurately estimate risk associated with current and past levels of chlorpyrifos exposure.

Proposed Approach:

Chlorpyrifos, like other OPs, inhibits AChE in the peripheral and central nervous system. This AChE inhibition leads to accumulation of acetylcholine and ultimately to neurotoxicity. Historically, AChE inhibition has been considered the primary mode of action for OPs and thus the most sensitive endpoint for purposes of human health risk assessment. In recent years, a

growing body of literature indicates that chlorpyrifos affect the developing the brain. The mode(s)/mechanism(s) by which chlorpyrifos causes such changes are unknown; many hypotheses have been posed including non-cholinergic modes of action (See review by Slotkin, 2006, Eaton et al. 2008). The dose-response relationships for changes in the developing brain are still unclear.

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 children, 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. Investigators associated with all three cohorts have evaluated the associations between OP levels (and/or their metabolites) in blood or urine and birth and neurodevelopmental outcomes.

These epidemiology studies have been performed using prospective methods such that exposure measures are taken before screening for potential outcomes. Chemical measures of blood and urine pesticide analytes are done by, or with, testing methods from the Centers Disease Control and Prevention (CDC). The studies are designed so that they can be compared to reference ranges in the National Health and Nutrition Examination Survey (NHANES) for diakyl phosphate metabolites (DAPs) and/or TCP in urine and chlorpyrifos in blood (See Attachment 1 for a summary of urinary and blood data from these studies). All three studies used well developed neurodevelopmental measures, which provide comparability. However, due to the nature of the exposure data collected, these studies have limited utility for deriving a reference dose/concentration for purposes of risk extrapolation. The SAP agreed with the Agency's preliminary conclusion "that there were limitations in the three epidemiological studies that precluded them from being used to directly derive the PoD or the uncertainty factor (p. 46, FIFRA SAP, 2008)."

It is, however, important for the Agency to evaluate the chlorpyrifos exposure measures from these studies to compare with current exposure levels. One of the key challenges in the new chlorpyrifos risk assessment is to relate the chlorpyrifos exposure associated with the blood and urine measures identified in the epidemiology studies to current chlorpyrifos exposure levels predicted for the general public and for agricultural workers. To accomplish this, the Agency plans to follow the advice of the SAP by following two lines of analysis.

- 1) *Bounding estimates.* The Agency will compare blood and urine levels reported in humans with blood and urine levels reported in rodent studies. These biological data can then be evaluated against outcome data in both rodents and humans. A variety of calculations can be made using central tendency estimates in addition to using values at the upper and lower ends of exposure distributions. In these calculations, the deliberate dosing studies in human subjects will provide a 'link' between the epidemiological studies and rodent

studies given that the administered dose is controlled and well-characterized in the experimental studies. This approach is depicted in Figure 1.

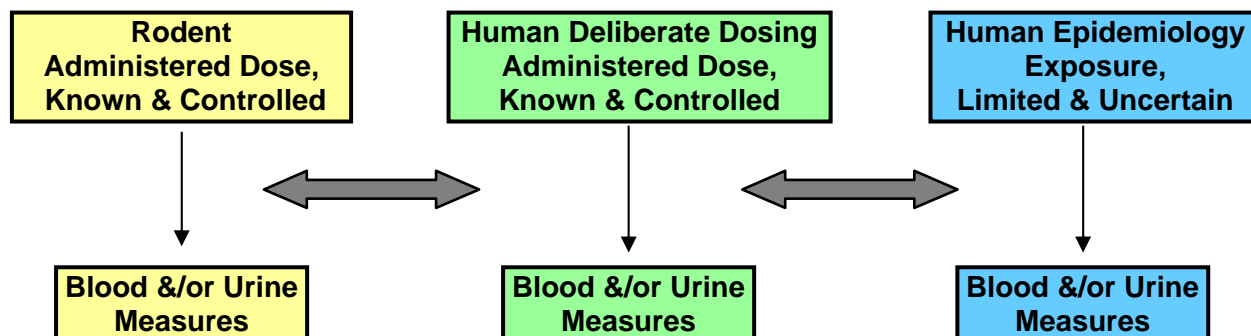


Figure 1. Conceptual approach for using the deliberate dosing studies to characterize the rodent and epidemiology studies

- 2) *Develop physiologically-based pharmacokinetic (PBPK) models.* As noted above, the SAP “encouraged the Agency to consider the use of a PBPK model to widen the application of these bounding data for current or potential human exposures and for the final reference dose or reference concentrations.” Several PBPK models have been published for chlorpyrifos (Timchalk et al, 2002a, 2007; Rigas et al, 2001; Knaak, et al, 2004; Georgopoulos et al, 2008).

In general, PBPK models are first developed for the rodent (particularly rat) then ‘scaled up’ to the human. PBPK models are typically supported by a combination of in vitro, in vivo, and in silico data. The chlorpyrifos PBPK models rely heavily on rat metabolism and AChE data and in vitro metabolism data from rodent and human tissues. The Timchalk model includes human urinary and blood data from both the Nolan et al. (1982, 1984) and Kisicki et al. (1999) studies. The human data provide information which informs the scaling procedure for converting the rat model into a human model.

The most extensive model(s) was developed by Dr. Charles Timchalk and co-workers at Pacific Northwest National Laboratory with funding from multiple sources including Dow AgroSciences and EPA.. It was first published in 2002 as an adult rat and human model (Timchalk et al., 2002a) and has been updated as more data have become available (Poet et al. 2003; Poet et al. 2004; Slikker et al. 2005; Timchalk et al. 2002b; Timchalk et al. 2003). 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. Lowe et al (2009) recently modeled late gestational exposures. The Agency is also aware of an effort by Drs. Dale Hattis and Robin Whyatt to develop PBPK model which includes a placental compartment for assessing tissue dosimetry to the fetus and which accounts for intra-species TK variability.

The Agency believes that the PBPK model(s) provide a valuable tool(s) which can be used to predict blood and urinary levels of chlorpyrifos and TCP following a variety of exposure scenarios. These predicted blood and urinary levels can then be compared to levels in the epidemiological studies in mothers and children and in population-based biomonitoring programs like NHANES.

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Attachment 1. Summary of Chlorpyrifos Blood Levels and TCP Urine Levels

Chlorpyrifos Blood Levels		
Study	Sample Size	Mean±SD and/or Range (ng/g)
Whyatt et al. (2003) (1998-2001) Mostly before Residential Phase Out	199 maternal blood; 211 cord blood	Maternal: 0.0048±0.0055 (ND-0.035) ^a
		Cord: 0.0047±0.0065 (ND-0.063) ^a
Whyatt et al. 2008 (presentation to EPA) (1998-2006) Pre and Post Phase out	425 maternal blood; 423 cord blood	Maternal: 0.0028±0.0044 (ND-0.035) ^a
		Cord: 0.003.0±0.0053 (ND-0.063) ^a
TCP Urinary Levels		
Study	Sample Size	(ng/ml or ug/L)
Berkowitz et al. 2003/2004 (NYC inner city cohort)	365	Maternal Spot Urine (approx 32 weeks of pregnancy): Median= 7.5 ug/L; 90 th percentile: 61.2 ug/L or 70 ug/g creatinine >11 ug/L associated with adverse birth outcomes in infants
Eskenazi et al. 2004,2007 Results (2000-2003) (CHAMACOS cohort)	445-485	Maternal Spot Urine: Median 3.3-3.54 ug/L Range: 0.2-56.1 ug/L
Lu et al. 2008	23	Children ages 3-11 years Mean spot urine is 5.1 ug/L; 95 th percentile of 14.7 ug/L (range of <0.2 to 32 ug/L
NHANES 1999-2002		Adults 18-59 years for 2001-2002 50 th Percentile: 1.9 ug/L Adults 95 th percentile: 11 ug/L
Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) (2005)	128	20 months-5.5 years (median 3.9 yrs), July 2000-March 2001 Spot Urine: Median: 5.3 ug/L Mean: 7.3 ug/L 95 th : 15.5 ug/L

(a) limit of detection is 0.5 to 1 pg/g, or 0.0005-0.001 ng/g.