Determination of the Appropriate FQPA Safety Factor(s) in the Organophosphorus Pesticide Cumulative Risk Assessment

Evaluation of Sensitivity and Susceptibility to the Common Mechanism of Toxicity, Acetylcholinesterase Inhibition

Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C.

June 10, 2002

PREFACE

The Food Quality Protection Act of 1996 (FQPA) directs EPA, in setting pesticide tolerances, to use an additional tenfold margin of safety to protect infants and children taking into account the potential for pre- and postnatal toxicity and the completeness of the toxicology and exposure databases. The statute authorizes EPA to replace this tenfold "FQPA safety factor" with a different FQPA factor only if reliable data demonstrate that the resulting level of exposure would be safe for infants and children.

Consideration of the FQPA safety factor provision applies to individual pesticide risk assessments and to cumulative risk assessments of multiple pesticides. In the December 2001 preliminary cumulative risk assessment on organophosphorus (OP) pesticides, EPA discussed and characterized the potential multiple sources of exposure to children, but did not present hazard information on the pre-and postnatal toxicity to these pesticides. EPA has revised the December 2001 preliminary cumulative risk assessment for OP pesticides. As part of that revision, an analysis was conducted on the sensitivity and susceptibility of infants and children to cholinesterase inhibition (the common mechanism of toxicity) caused by OP pesticides.

This report addresses the need for application of uncertainty factors and/or retention of the additional 10X FQPA safety factor for the protection of infants and children. The pertinent hazard and exposure information is presented to evaluate the safety of the OP pesticides for infants and children, and thus discusses: the adequacy of the exposure assessment; whether data deficiencies are present that should be addressed by application of uncertainty factors, which are used to address the FQPA safety factor provision's expressed concern as to the "completeness of the data with respect to . . . toxicity to infants and children . . . ;" and the concern for sensitivity and susceptibility to cholinesterase inhibition.

This report is incorporated in the revised cumulative risk assessment of OP pesticides as a separate section. It is also presented here as a stand-alone document for review in June 2002 by the FIFRA Scientific Advisory Panel.

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ACRONYMS

AChE	Acetylcholinesterase
CAG	Cumulative Assessment Group
ChE	Cholinesterase
ChEI	Cholinesterase Inhibition
CNS	Central Nervous System
CSFII	USDA's Continuing Survey of Food Intakes by Individuals
DCI	Data Call-In
DNT	Developmental Neurotoxicity
EPA	U.S. Environmental Protection Agency
EXAMS	EXposure Analysis Modeling System
FCID	Food Commodity Intake Database
FDA	U.S. Food and Drug Administration
FIFRA	The Fungicide, Insecticide, and Rodenticide Act
FQPA	Food Quality Protection Act of 1996
GD	Gestation Day
HED	U.S. EPA's Health Effects Division
LD	Lactation Day
MNCPES	The Minnesota Children's Pesticide Exposure Study
MOE	Margin-of-Exposure
MTD	Maximum Tolerated Dose
NERL	U.S. EPA's National Exposure Research Laboratory
OP	Organophosphorus or Organophosphate
OPP	U.S. EPA's Office of Pesticide Programs
PDP	USDA's Pesticide Data Program
PND	Postnatal Day
PRZM	Pesticide Root Zone Model
RBC	Red Blood Cell
RPF	Relative Potency Factor
SAP	FIFRA Scientific Advisory Panel
UF_{Db}	Database Uncertainty Factor
USDA	U.S. Department of Agriculture
USEPA	U.S. Environmental Protection Agency

I. INTRODUCTION

There is currently a significant focus on the potential susceptibility and increased sensitivity of infants and children to toxic effects of chemicals (see National Resource Council's 1993 report, *Pesticides in the Diets of Infants and Children*). The Food Quality Protection Act of 1996 (FQPA) instructs the U.S. Environmental Protection Agency (EPA or the Agency), in making its "reasonable certainty of no harm" finding, that in "the case of threshold effects, . . . **an additional tenfold margin of safety** for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account **potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children**." Section 408 (b)(2)(C) further states that "the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children."

A. Guidance Used for Consideration of the FQPA Safety Factor

EPA's Office of Pesticide Programs (OPP) has recently released revised guidance addressing application of the FQPA safety factor provision in risk assessments for individual pesticide chemicals (USEPA, 2002a). Additionally, OPP has prepared a separate guidance document addressing the application of the FQPA safety factor provision in the context of cumulative risk assessments for two or more pesticides sharing a common mechanism of toxicity (USEPA, 2002b; released February 28, 2002 for a 60-day comment period). Both FQPA safety factor guidance documents (USEPA, 2002a,b) were used to provide general guidance on applying traditional uncertainty factors and on implementing the FQPA safety factor provision for the cumulative risk assessment of organophosphorus (OP) pesticides. In implementing the FQPA safety factor provision, key considerations in a cumulative risk assessment are:

- ❑ determining the completeness of the data with respect to effects that may occur in the young due to the common mechanism of toxicity;

- ❑ evaluating the degree of concern regarding the potential for pre- and postnatal effects associated with the common mechanism of toxicity and determining the residual uncertainties not addressed by application of traditional uncertainty factors to account for deficiencies in the toxicity data; and

- ❑ determining the completeness of the exposure database for all pertinent pathways of exposure to OP pesticides.

B. Scope of Analysis on Sensitivity and Susceptibility

Single-chemical risk assessments should generally be conducted for each member of a common mechanism group before a cumulative assessment is attempted. Thus, previous determinations have been made whether to retain or replace the FQPA 10X safety factor for the individual pesticide members of the OP cumulative risk assessment group. These FQPA safety factor decisions should be revisited, however, in the cumulative risk assessment process because they are based on broader considerations of potential toxic effects in the young (*e.g.*, teratogenicity, carcinogenic effects) that may not relate to the common mechanism of toxicity. A cumulative risk assessment differs from the single-chemical risk assessment both in focus and purpose. The cumulative risk assessment of the OP pesticides is based on their ability to target and inhibit the enzyme acetylcholinesterase (AChE) in nerve tissue, in other words, the common mechanism of toxicity for which these pesticides are grouped. Thus, decisions on the FQPA safety factor for the cumulative assessment group (CAG) reflect considerations that pertain to the common effect and the common mechanism of toxicity.

Several years ago, the International Life Sciences Institute/Risk Sciences Institute (RSI) convened an expert panel to address whether the OP pesticides act by a common mechanism of toxicity (Mileson *et al.*, 1998). Although some OP pesticides may act by several different neurotoxic mechanisms through interaction with other esterases and nonesterase targets (for review see Pope and Liu, 2001), there are insufficient data to support subgrouping of the OP pesticides based on other actions operating instead of, or in addition to, the inhibition of AChE. It should be pointed out that these other mechanisms are considered in the individual risk assessments of the OP pesticides when there is sufficient available information. For example, in evaluating the susceptibility of the young to chlorpyrifos, OPP considered data that showed effects on the developing rat brain such as structural defects and changes in macromolecular synthesis, neurotransmitter levels, and cell signaling. Although these other neurodevelopmental mechanisms are considered in the single chemical assessment, they will only be considered in the cumulative analysis as they relate to AChE inhibition. Because AChE inhibition is the mechanism of toxicity and precursor event to toxicity, functional effects in the young that result from the inhibition of AChE activity should not occur at doses lower than those causing AChE inhibition.

II. HAZARD ASSESSMENT: SENSITIVITY AND SUSCEPTIBILITY[1]

The hazard assessment, below, considers the potential pre- and postnatal developmental effects that may be associated with the inhibition of AChE, the comparative AChE inhibition between adults versus the immature animal, and the completeness of toxicity data on AChE inhibition in young animals.

A. Role of Acetylcholinesterase in Neurodevelopment

AChE is the enzyme that hydrolyzes the neurotransmitter acetylcholine at cholinergic synapses and neuromuscular junctions. The inhibition of AChE leads to accumulation of synaptic acetylcholine, overstimulation of postsynaptic cholinergic receptors and consequent signs of neurotoxicity or cholinergic toxicity. It has been suspected, however, for more than 25 years that AChE may have an extrasynaptic, noncholinergic role during development (*e.g.*, Karczmar *et al.*, 1973; Drews, 1975). Recent research indicates that the roles of AChE during development center around neurogenesis, cell adhesion and possibly stress response (*e.g.*, Layer and Willbold, 1995; Grisaru *et al.*, 1999; Bigbee *et al.*, 1999; Brimijoin and Koenigsberger, 1999). Moreover, the widespread expression of AChE is often mirrored by the expression of acetylcholine, which is involved with basic developmental processes such as mitosis, cell-to-cell contact, cell adhesion, cell differentiation, and organization of the cytoskeleton (reviewed in Wessler *et al.*, 1999; Lauder and Schambra, 1999).

Both AChE and acetylcholine are highly conserved molecules which have multiple roles in the developing nervous system as well as extraneuronal functions. Because AChE controls acetylcholine levels in neuronal as well as extraneuronal tissues and blood (*e.g.*, Wessler *et al.*, 1998; Fujii and Kawashima, 2001; Kirkpatrick *et al.*, 2001) and because AChE activity is more commonly measured as compared to acetylcholine levels, most of the work reviewed below concentrates on changes in AChE activity rather than acetylcholine levels. One may assume, however, that as mentioned above, a decrease in AChE activity should also increase acetylcholine concentration. Changes in the structure, activity or level of these neuromodulators, AChE or acetylcholine, may elicit novel effects on the developing brain. It is not known to what extent neuronal AChE needs to be altered to have adverse effects on the developing brain, nor is it known what adverse

[1]The term susceptibility is used qualitatively to indicate unique effects (*e.g.*, a different pattern of effects of concern) in the young. The term sensitivity is used to refer to quantitative susceptibility, or to quantitatively indicate effects of a type similar to those seen in adults, but which occur at doses lower than those causing effects in adults.

effects on neurodevelopment may result from AChE inhibition. Nevertheless, because of the potential developmental role of AChE, it is reasonable to consider the evidence for whether inhibition of AChE in the developing nervous system may affect neural development.

In vitro work has shown that some OP compounds can inhibit neurite outgrowth, but enzyme inhibition does not appear to correlate completely with inhibition of outgrowth (Dupree and Bigbee, 1994; Layer *et al.*, 1993, Bigbee *et al.*, 1999). Inhibition of neurite outgrowth is compound-specific, as some compounds inhibit AChE activity but do not inhibit neurite outgrowth. It is now accepted that the cell adhesive function of AChE is mediated by a peripheral anionic site located at the rim of the 20 Å gorge, a site distinct from the catalytic site located at the bottom of that gorge (Johnson and Moore, 1999; Sternfeld *et al.*, 1998). OP inhibitors bind to the catalytic site; little is known about prerequisites for binding to the peripheral anionic site mediating cell adhesiveness. Perhaps some OPs bind specifically to that site or perhaps some OPs can perturb the function of that site when bound to the catalytic site (*e.g.*, Bigbee *et al.*, 1999).

In any event, AChE inhibition does not necessarily predict perturbations of neuronal differentiation. It is possible to create fruit flies (Greenspan *et al.*, 1980) or mice (Xie *et al.*, 2000) that do not produce AChE because they have no gene for AChE. In fruit flies, this is a lethal mutation, but in mice the absence of AChE is only lethal to approximately 25% of the homozygous fetuses *in utero*. At birth, the surviving homozygous animals appear overtly normal, but fail to develop normally and usually die by day 21 unless care is taken to provide their nutritional needs, in which case they may live to adulthood. The authors speculate that the animals survive because butyrylcholinesterase assumes many of the biochemical functions of the absent AChE. As with any study with knockout mice, the phenotype must be interpreted with caution as compensation may occur during development that would not mimic AChE inhibition during development.

Is there evidence that exposure to OP pesticides pre- or postnatally perturbs neurodevelopment? Some animal studies using prenatal exposures show effects on neurodevelopment, while other studies do not show any effect. In general, the literature shows that high levels of dosing of an OP during gestation (*e.g.*, affecting maternal weight gain) will tend to be embryotoxic (*i.e.*, lethal). More subtle effects may be noted at lower doses if other neurodevelopmental specific tests are employed. For example, the offspring of mice receiving diazinon during gestation showed developmental delays and abnormal endurance and coordination at doses of 0.18 or 9 mg/kg/day (Spyker and Avery, 1977). Malathion or dicrotophos showed dose and age-related abnormalities (assessed histologically) of nervous and extranervous system development in one-, two-, and three-day-old chick embryos (Wyttenbach and Thompson, 1985; Garrison and Wyttenbach, 1985) An *in vivo* study of malathion, however, showed no teratological effects in rabbits dosed from day 7 to day 12 of gestation (100 mg/kg; Machin and McBride, 1989); note that this study did not include any detailed assessment of nervous system tissues. Fetal brains of rats given chlorpyrifos repeatedly during late gestation show abnormalities

in neuronal migration and other biochemical endpoints (Lassiter *et al.*, 2002; Qiao *et al.*, 2002). Gestational exposure (day 6 to day 15) to tribufos, oxydemeton-methyl, azinphos-methyl, fenamiphos, isofenphos or fenthion at doses that produced 20-50% maternal brain cholinesterase (ChE) (ChE is used when there was no distinction between butyryl- or acetyl-cholinesterase in the experimental procedure) inhibition showed no embryotoxicity or teratogenicity; neurodevelopment was not assessed (Astroff and Young, 1998). Although the authors conclude that gestational dosing with these compounds caused "no effect on fetal ChE," this activity was not assessed until five days after the last dose, a time that is not optimal for assessing AChE inhibition in fetal tissues (Lassiter *et al.*, 1998; Michalek *et al.*, 1985).

Rats given OP pesticides postnatally may show abnormal nervous system development. In a series of papers exploring the neurotoxicity of postnatally administered chlorpyrifos, many changes were noted (*e.g.*, RNA levels, transcription factor expression, disruption of catecholaminergic and cholinergic pathways) (Johnson *et al.*, 1998; Crumpton *et al.*, 2000; Dam *et al.*, 1999), resulting in persistent biochemical and behavioral changes long after the dosing ceased (Dam *et al.*, 2000; Slotkin *et al.*, 2001a,b; Levin *et al.*, 2001; Slotkin *et al.*, 2002). Other studies in which chlorpyrifos was administered to the dam so that the pups received their dosage only through the milk were largely negative (Breslin *et al.,* 1996; Deacon *et al.*, 1980; Maurissen *et al.,* 2000), although the endpoints examined were not as targeted and discriminating as those used by the Slotkin laboratory. The relationship of these neurodevelopmental changes to ChE inhibition is unclear because many studies are lacking correlative ChE activity, thus making it difficult to draw firm conclusions. In the few prenatal studies where ChE activity was assessed, however, few of these effects occur at dose levels that do not inhibit ChE activity in the fetal brain, and probably none of these effects occur in the absence of ChE inhibition in maternal tissues. In both the studies assessing prenatal effects of chlorpyrifos, effects on brain development were noted at dosages (1 mg/kg/day) that did not inhibit fetal brain ChE (Lassiter *et al.*, 2002; Qiao *et al.*, 2002), but would be predicted to show inhibition of maternal blood and brain ChE activity (Maurissen *et al.*, 2000). In postnatal studies, there are no reports of effects in the absence of ChE inhibition. In some cases, this assertion is made by the authors, but the authors fail to ascertain that the ChE measurements were taken at the time of peak effect. Often the measurements are taken 24 hours after the last dose, rather than assessing ChE activity during the entire dosing period. Thus, it is reasonable to assume that adverse neurodevelopmental outcomes that are a result of the inhibition of ChE should not occur at doses that do not inhibit ChE. Because, however, the cumulative risk assessment is based on adult brain ChE data, it is important to address the age-related sensitivity of ChE inhibition in the adult versus the young animal. The available studies are reviewed below.

B. Differential Sensitivity of the Young Compared to the Adult

Although reports of increased sensitivity of the young following exposure to OP pesticides date back over two decades, it is the work that has emerged recently that provides a better basis for understanding the issues concerning the sensitivity of the young to ChE inhibition. This understanding comes from recently generated chemical-specific data in young animals on ChE activity, as well as generic human and animal studies on the biological and biochemical parameters involved in age-dependent sensitivity. The current state of the knowledge is summarized and discussed below.

1. Human Incident Information

There are reports of symptoms associated with cholinergic toxicity due to accidental acute exposures. A 1999 review based on pesticide-related exposures (excluding cases of exposure to multiple products, attempted suicides, malicious intent, and confirmed non-exposure) examined Poison Control Centers Data from 1993 through 1996 (USEPA, 1999). Of the exposures that occurred in a residential setting 16% were due to OP pesticides. The review of the residential pesticide exposure concluded:

Organophosphate pesticides pose a greater hazard from accidential acute exposure than do other pesticides, especially for children under six years-of-age. Children were three times more likely to be hospitalized, five times more likely to be admitted for critical care, and four times more likely to have experienced a major medical outcome or death than if exposed to some other pesticide.

In this review of residential exposures, there were 24,889 exposures reported in children under the age of six, 5,080 exposures among children six to 19 years-old, and 32,087 exposures among adults. Of those cases with medical outcomes determined, children under age six were 22% more likely to experience a life-threatening or fatal outcome as a result of their exposure than adults or children six to 19 years-old. Additionally, based on the Centers for Disease Control mortality data (see http://wonder.cdc.gov/mortsqu.shtml), the ratio for death in young children exposed to OP pesticides was 3.3 times higher than in adults.

These data show that there is more potential for harmful exposures in young children than in older age groups, but they do not necessarily demonstrate an increase in the sensitivity of young children. There is a possibility that young children may be exposed to higher doses on a body weight basis compared to adults (from spills, ingestion, inhalation) because they are ignorant of the hazard, and not because of differences in sensitivity based on age to the effects of these pesticides. Furthermore, the human data on children come from accidental exposures to these pesticides that are associated with acute poisoning resulting

in significantly higher blood, tissue, and urine concentrations of these chemicals compared to exposures that humans would normally encounter in the food or the environment.

Because of the reasons stated above, it is difficult to draw conclusions from human incident data on the sensitivity of the young compared to adults. The animal literature below allows for evaluations of age-dependent sensitivity.

2. Laboratory Animal Studies

Some studies are available in the open literature that have evaluated ChE inhibition following *in utero* or lactational exposures to OP pesticides, as well as dosing of young animals. EPA issued a Data Call-In (DCI) on September 10, 1999 for adult and developmental neurotoxicity (DNT) studies on the OP pesticides, and as part of the DNT protocol, measures of brain, red blood cell (RBC), and plasma ChE activity in dams and pups were required to characterize comparative levels of inhibition at the time of peak effect. However, very few DNT rat studies have been submitted to the Agency.[2] In addition to studies on OP pesticides that allows a comparison of the differential in response to ChE inhibition between adult and immature rats, several recent published studies provide an important perspective on the underlying basis for observed increased sensitivity. The analyses below will focus on differences in ChE inhibition between fetal, neonates, and juvenile rats compared to adults.

a. Differential Sensitivity Following Gestational/Lactational Exposure

Fenamiphos, tribufos, trichlorfon, and oxydemeton-methyl were evaluated for ChE inhibition in a rat multigeneration reproductive feeding study (Astroff *et al.*, 1998; discussed in Sheets, 2000). Dams were treated with these OP pesticides via the diet during gestation and continuing throughout the lactation period. Pups are assumed to be exposed due to consumption of feed at about 14-21 days-old.

Plasma and RBC ChE activity were measured in the adults during the premating phases of both generations following eight weeks of exposure to each of the OP pesticides, and again at termination when brain ChE activities were measured. Separate contingents of postnatal rats were evaluated for plasma, RBC, and brain ChE activity on lactation day (LD) 4 and on LD21. The effects found on LD4 could be due to gestational and lactational exposure, whereas the results on LD21 may reflect exposure through the milk

[2]Out of the 30 OP pesticides included in the December 2001 preliminary cumulative assessment, DNT studies have only been submitted for chlorpyrifos, dimethoate, malathion, methyl parathion, methamidophos, and tribufos. The DNT studies submitted for dimethoate, malathion, and methyl parathion also included comparative ChE activity. These studies investigated ChE activity in adult and immature rats following either acute or repeated dosing. A review of the chlorpyrifos DNT study was completed in 1999, and reviews of the dimethoate and malathion DNT studies have recently been completed. The DNT studies for tribufos, methamidophos, and methyl parathion are currently under review, although a review has been completed on the ChE data for malathion and methyl parathion. It should be pointed out that the DNT studies on methamidophos and tribufos are feeding studies in which the pups were not directly dosed, and thus the pups were presumed to be exposed only *in utero* and during lactation; no comparative ChE data have been submitted for methamidophos and tribufos.

and some exposure through the diet as pups begin consuming feed in the late lactational period, in other words, postnatal days (PND)14-21. Each study consisted of a control and three dose groups. The highest dose level was selected based on parental toxicity.

Toxicity (reduced body weights or viability) in the young was not apparent until there were significant maternal effects (decreased body weights and food consumption) and substantial ChE inhibition in the blood and brain of the parental animals. In fact, the adult animals were more affected than the young in this study. Although young rats, when exposed to these OPs *in utero* and via lactation, do not appear to exhibit more ChE inhibition than is found in maternal tissues, the dose that may be absorbed by the fetus and adult is unknown. Thus, conclusions can not be reached about the relative sensitivity of fetuses versus dams to ChE inhibition.

Maternal and fetal ChE inhibition were evaluated following maternal exposure to **azinphos-methyl, fenamiphos, fenthion, isofenphos, tribufos, and oxydemeton-methyl** in a prenatal developmental toxicity study in rats (Astroff and Young, 1998). These pesticides were administered to the dams by gavage on gestation days (GD) 6-15. Maternal ChE activity (brain, RBC, plasma) was measured onGD16 and 20, and fetal brain ChE activity was measured on GD20. The dose levels for these studies were selected such that maternal ChE inhibition at the highest dose tested was greater than 20%. At the highest dose tested on GD16 (in plasma [except for azinphos-methyl], RBC [except for fenamiphos], and brain [except for fenamiphos], and on GD20 (in plasma [only for fenthion], RBC [except for aniphos methyl], and brain [except for fenamiphos]), maternal ChE was significantly inhibited. However, no remarkable brain ChE inhibition was observed in fetuses at any dose on GD20.

The effect of treatment with **chlorpyrifos** on ChE activity was compared in dams and fetuses by Mattsson *et al.* (1998; 2000). Pregnant Sprague-Dawley rats were administered chlorpyrifos by gavage at doses of 0, 0.3, 1.0, or 5.0 mg/kg/day on GD6-20. The magnitude of brain, plasma, and RBC ChE inhibition in the fetus on GD20 was found to be less than or equal to that observed in dams. At 5.0 mg/kg/day, ChE activity in fore- and hindbrain of the dams on GD20 was inhibited by 76.0 and 86.7%, respectively, and by 58.8% in fetuses. At 1.0 mg/kg/day, brain ChE activity in fore- and hindbrain was inhibited in dams by 7.8 and 8.0% (statistically significant at $p \leq 0.05$ or 0.01), respectively; there was no statistically significant depression of brain ChE activity in fetuses. In another study of the comparative ChE inhibition between dam and fetus with chlorpyrifos, Lassiter *et al.* (1998) concluded that the fetal brain ChE inhibition was less than the maternal brain ChE inhibition during repeated dosing primarily because the fetal brain tended to recover more completely between doses than the maternal brain ChE. When dams were given a single dose, both maternal and fetal brain ChE appeared to be depressed to the same degree, but when subjected to a repeated dosing

regimen, the fetal brain showed less inhibition probably because of the higher rates of new synthesis or more rapid turnover of inhibited molecules of ChE in the fetuses compared to the adult. In two different studies which compared the tissue burden of chlorpyrifos and metabolites in dam and fetus, one group (Mattsson *et al.*, 1998, MRID 44648102, Mattsson *et al.*, 2000) found lower blood concentrations of chlorpyrifos in the fetus as compared to the dam, whereas another group (Hunter *et al.*, 1999) found three times more trichloropyridinol (a metabolite of chlorpyrifos) in the fetal brain as compared to the maternal brain. Trichloropyridinol (TCP) can either be produced as a by-product of a toxic action (*i.e.,* TCP is the leaving group when chlorpyrifos-oxon binds to ChE) or of a detoxification action (*e.g.,* TCP can be produced when chlorpyrifos-oxon is catalyzed by PON1).

Results of a recently submitted DNT study with **dimethoate** indicated that treatment by gavage of dams with the pesticide induces equal or less inhibition of ChE in the fetus compared with the dams (Meyers, 2001; MRID 45529702). Treatment of dams with 0.5 mg/kg/day of dimethoate during GD6-20 induced statistically significant but marginal ChE inhibition (10%) in brain tissue of both adult and fetal rats. The responses at 3 mg/kg/day (the highest dose tested) indicated less brain and RBC ChE inhibition in fetuses (33% and 31%, respectively) compared with dams (60% and 58%, respectively). Measurements of ChE inhibition were also conducted on four-day-old pups that were exposed to dimethoate *in utero* from GD6 to GD20, but not directly exposed postnatally. At 3.0 mg/kg/day, brain and RBC ChE activity was inhibited by 13% and 17% in the PND4 pups.

A DNT study performed with **malathion** (Fulcher, 2001; MRID 45566201) also showed that there was less effect on ChE activity (measured at GD20) in fetuses than in dams that had been treated by gavage with the pesticide during GD6-GD20. At the highest dose examined (150 mg/kg/day), RBC ChE was inhibited by 19% in fetuses and by 51% in the dams. No effects on brain ChE activity were observed in either the fetuses or dams at that dosage. At PND4, at which time the only exposure to malathion could be through milk, ChE activities in treated pups were comparable to controls.

ChE data that were recently submitted to the Agency (Beyrouty, 2002b; MRID 45656501), supplemental to a DNT study on **methyl parathion**, demonstrated that treatment of dams by gavage from GD6-20 induced more ChE inhibition in the brain of dams than in the fetuses. Analyses of brain tissue at GD20 showed that ChE activity was inhibited by 31% in dams at a dose of 0.60 mg/kg/day (the highest dose tested), while there was no brain ChE inhibition in their fetuses. At the same dose, RBC ChE inhibition was 58% in dams and 22% and 18% in male and female fetuses, respectively. In PND4 pups, ChE was not inhibited in any compartment.

In summary, results of studies with fenamiphos, tribufos, trichlorfon, oxydemeton-methyl, chlorpyrifos, methyl parathion, dimethoate, malathion,

and azinphos-methyl show that treatment of pregnant dams with an OP pesticide during gestation induces more ChE inhibition in the dams than in the fetus. Data from these studies also show that the newborn (one- to four-day-old pups), when only exposed *in utero* or possibly through the milk, also has less inhibition of ChE than the maternal, adult rat. The lack of similar levels of ChE inhibition in fetuses or neonates relative to adults may be due to the fetuses receiving a lower dose of these OP pesticides compared to their dams because of pharmacokinetic differences, such as a lower dose being transferred to the fetus through the placenta or to the neonate through the milk than is received by the dam directly in the diet. A lower response in the immature animal may also be due to the increased synthesis or more rapid turnover of inhibited molecules of ChE in the fetal brain compared to the adult (Lassiter *et al.*, 1998; Mortensen *et al.*, 1998).

b. Differential Sensitivity Following Direct Postnatal Exposures

Neonatal, juvenile, and adult rats show differential sensitivity to ChE inhibition following an acute gavage treatment with **chlorpyrifos** (Pope, 2001a). When rats from each age group were administered chlorpyrifos at 0.5 times the LD_{10} (7.5 mg/kg, neonates; 23.5 mg/kg, juveniles; 68 mg/kg, adults), peak ChE inhibition in the cortex (estimated from Figure 11 of the report) was 70% (neonates), 65% (juveniles), and 68% (adults). Thus, based on similar magnitudes of peak ChE inhibition at 0.5 of a LD_{10} dose and considering the differentials in the 0.5 LD_{10} doses, neonates were shown to be about threefold more sensitive than juveniles and about ninefold more sensitive than adults. In another study by Moser *et al.* (1998) a single gavage dose of 20 mg/kg produced 89% and 91% (males and females) brain ChE inhibition in PND17 pups, compared to 39% and 36% (males and females) inhibition in adults (*i.e.*, about a twofold difference in relative sensitivity).

Chlorpyrifos produces a minimal difference in ChE inhibition in neonatal rats compared to adult rats following repeated dosing (14 treatments by gavage during PND7 to PND21). Based on ED_{50} levels (adults, 3.3 mg/kg/day and neonates, 2.2 mg/kg/day), the difference in the response of neonates to brain ChE inhibition compared to adult males is a 1.5-fold increase (Zheng *et al.*, 2000).

Similar results were reported in a recent study in which neonatal and adult rats were administered chlorpyrifos by subcutaneous injection (Liu *et al.*, 1999). Neonatal (seven-day-old) pups and adults were administered 0, 5, or 10 mg/kg/day for seven or 14 days and sacrificed for ChE measurements one day after the final dose. At seven days, inhibition of ChE activity in the cortex and striatum of the neonates was 62 and 65%, respectively, compared with 50 and 55% in adult animals. Following 14 days of treatment, ChE inhibition in the cortex and striatum of neonates was 60 and 65%, respectively and 65% in both of these tissues from adult animals.

Diazinon. A recent abstract by Moser and coworkers (Padilla *et al.*, 2002) reported an increased sensitivity to ChE inhibition for diazinon when PND17 pups were given a single oral dose (via gavage) of 75 mg/kg (75% brain ChE inhibition) compared to adult rats (38% brain ChE inhibition). This observation was correlated with detoxification by carboxylesterases and A-esterases (as discussed later in Section II.C.). There are no data available on the effects on ChE activity following repeated dosing of neonates or young adults with diazinon.

Dimethoate. In a recent DNT study (Meyers, 2001; MRID 45529702), dimethoate was evaluated for ChE activity in plasma, RBC, and brain following acute exposures and repeated dosing (subacute exposures) to 0.1, 0.5, and 3 mg/kg/day of dimethoate. Dimethoate was given by gavage to pregnant rats GD6 through LD10 (LD10; equivalent to PND10); their pups were treated by gavage from PND11 through PND21. Plasma, RBC, and brain ChE was measured at GD20 (dams and fetuses), PND4 (pups only), and PND21 (pups only). ChE activity was also measured following an acute dose of dimethoate to additional groups of young adult and PND11 rats. In general, there was no striking difference in sensitivity to dimethoate-induced brain or plasma ChE inhibition between males and females of either adults or pups following acute or repeated treatment.

Acute (single dose) treatment with dimethoate of adult male and female rats and 11-day-old offspring with 3 mg/kg/day induced statistically significant, treatment related ChE inhibition in brain or RBC. At that dose, brain ChE inhibition in adult male and female rats was 12% and 14%, respectively, and in day 11 male and female offspring 17% and 18%, respectively. At 3 mg/kg/day, RBC ChE inhibition was greater in adult females than in adult males (26% versus 17%) and there was no statistically significant depression of RBC ChE activity in PND11 offspring.

Repeated dosing (11 doses) with 0.5 mg/kg/day dimethoate induced statistically significant but marginal brain ChE inhibition (10-13%) in both sexes of adult and 21-day-old rats. The response is likely due to treatment with the chemical because of the positive finding in both sexes of both age groups and because data from GD20 dams also showed an effect at 0.5 mg/kg/day. At the 3 mg/kg/day dose level, brain ChE inhibition was substantial in both adults (up to 58%) and 21-day-old offspring (up to 45%). In the repeated dosing study, a small, but statistically significant, difference in brain ChE inhibition was found at the low dose (0.1 mg/kg) between adults and pups (GD20, PND4 and PND21). As the dose was increased, this differential was not found at the high dose 3.0 mg/kg (see Table 1).

Because dimethoate does not show age-dependent sensitivity (discussed above), it is reasonable to assume that its oxon–omethoate–will also not show a differential toxicity in adults versus pups. Unlike acephate (discussed later), the parent compound–dimethoate–has been characterized for ChE inhibition in the young animals compared to adults.

Malathion. Recently submitted ChE data (supplemental to a DNT study) (Fulcher, 2001; MRID 45566201) clearly demonstrate a differential sensitivity to inhibition of the ChE enzyme in immature animals compared to adult rats treated with acute or repeated exposure to malathion. In this study, pregnant rats were administered malathion by gavage from GD6 through LD10; gavage dosing of their pups was then continued from PND11 through 21. Plasma, RBC, and brain ChE was measured at GD20 (dams and fetuses), PND4 (pups only), and PND21 (pups only). ChE activity was also measured in additional groups of young adult and immature (PND11) rats that had been administered a single (acute) dose of malathion. The dose levels were 5, 50, 150 mg/kg/day in the repeated dosing studies, and 5, 50, 150, and 450 mg/kg in the acute studies.

Following an acute dose of malathion, brain ChE was inhibited in PND11 pups at 150 mg/kg (44% in males and 48% in females) and at 450 mg/kg (84% in males and 81% in females), while brain ChE was not affected in young adults at either of those doses. At 450 mg/kg, however, RBC ChE was inhibited in both young adults (25% in males and 17% in females) and PND11 pups (72% in males and 61% in females).

Repeated dosing of malathion at 150 mg/kg/day from PND11 through PND21 (11 days of treatment) produced a marked inhibition of plasma (24-32%), RBC (67-68%), and brain (16%) ChE compared to controls. For dams, 14 days of treatment at 150 mg/kg/day resulted in RBC ChE inhibition (51%), but no inhibition of plasma or brain ChE. Similarly, for young adult rats that were treated for 11 days with 150 mg/kg/day malathion, RBC ChE was inhibited 43% in males and 48% in females, while plasma and brain ChE were not affected. At 50 mg/kg/day, plasma (19%) and RBC (34-39%), but not brain, ChE activity was inhibited in the PND21 offspring, while in dams and young adults, only RBC ChE (19-20%) was inhibited. No effects on ChE activity were seen at 5 mg/kg/day for dams or young adults. In PND21 offspring, however, RBC, but not plasma or brain, ChE was inhibited (17% in males, 15% in females).

Methamidophos was evaluated for age-related differences in ChE inhibition by Moser (1999). Comparisons for brain and blood ChE activity were made between PND17 and adult rats following acute oral doses of 1, 4, or 8 mg/kg. The dose response curves for ChE inhibition were quite similar between pups and adult rats. ED_{50} values for brain ChE inhibition in PND17 and adult rats were approximately 3.3 and 3.0 mg/kg/day, respectively. The ED_{50} values for blood ChE inhibition were 2.5 (PND17) and 2.2 (adults)

mg/kg/day.

Although acephate is metabolized to methamidiphos, it is not possible to determine, based on available data, whether acephate would show comparable responses in adult and young rats. This is because acephate, the parent compound, has not been evaluated for comparative ChE activity in young versus adult animals. In rats, only a small portion of acephate is metabolized in mammals to methamidiphos (5%) (Warnock, 1973; MRID 00014219). Furthermore, it is unknown to what extent the parent chemical may induce ChE inhibition or to what extent the parent chemical may alter the effects of methamidiphos on ChE activity in the adult or young rat.

Treatment of neonatal, juvenile, and adult rats with a single gavage dose of **methyl parathion** induces a differential response among the age groups in ChE inhibition in the brain (cortex) (Pope, 2001a). Treatment with methyl parathion at doses of 1.0 mg/kg (neonates), 2.05 mg/kg (juveniles), or 7.3 mg/kg (adults) induced similar magnitudes of peak ChE inhibition (60%-70%, estimated from Figure 14 of the report). Based on a comparison of the doses that induced similar levels of ChE inhibition, neonates are 2.5-fold more sensitive than juvenile rats and about sevenfold more sensitive than adult rats to methyl parathion induced ChE inhibition.

Methyl parathion was investigated by Liu *et al.*, (1999) for ChE activity and other neurochemical effects after repeated dosing in postnatal and adult male rats. Adult and postnatal rats (eight-day-old) were treated with methyl parathion subcutaneously at 1.5 mg/kg/day or 3.0 mg/kg/day for either seven or 14 consecutive days. Brain ChE activity was measured in the cortex and in the striatum one day after seven days of dosing or eight days after 14 days of dosing. Brain ChE activity was more reduced in postnatal pups compared to adults. Following seven days of dosing at 1.5 mg/kg/day, neonates showed 62 and 75% ChE inhibition in the cortex and striatum, respectively, compared to 25 and 30% in the adult male rats. In neonates treated subcutaneously with methyl parathion for 14 days, ChE activity was inhibited in the cortex by 65% and in the striatum by 80%. ChE activity was inhibited in adult rats by 40% (cortex) and by 50% (striatum).

A recently submitted DNT study on methyl parathion with supplemental ChE data (Beyrouty, 2002b; MRID 45656501) demonstrated a differential sensitivity of immature versus adult rats to ChE inhibition following acute or repeated exposure. The protocol for this study was similar to that used for the DNT ChE studies conducted for dimethoate and malathion. Methyl parathion was administered by gavage to pregnant rats from GD6 through LD10 at doses of 0.03, 0.11, 0.3, and 0.6 mg/kg/day. Pups from these litters were then administered methyl parathion by gavage from PND11-21. Plasma, RBC, and brain ChE was measured at GD20 (dams and fetuses), PND4 (pups only), and PND21 (pups only). Additional groups of young adult and PND11 rats were dosed acutely with methyl parathion (at doses of 0.03,

0.11, 0.3, and 0.6 for adults and doses of 0.03, 0.11, 0.3, and 1.0 for pups), and ChE activity was measured. Following acute exposures of 0.3 mg/kg, ChE was inhibited in brain (15-18%), RBC (20-31%), and plasma (25%) in PND11 pups; no inhibition was observed in any compartment for adults.

Repeated dosing of PND11 to PND21 pups (which had also been exposed *in utero* and via lactation) also showed an increased sensitivity of neonates compared with adult rats to treatment with methyl parathion. At 0.3 mg/kg/day, ChE activity was inhibited in PND21 pups (brain 26-29%, RBC 62-65%, and plasma 24-31%). In dams treated with the same dosage from GD6 to GD20 (*i.e.*, 14 days of treatment), ChE inhibition was seen in brain (9%) and RBC (35%), but plasma ChE was not affected. In adult rats treated with 11 repeated doses of 0.3 mg/kg/day methyl parathion, ChE inhibition was seen in RBC (30% in males and 35% in females) and plasma (25% in males), but there was no inhibition of brain ChE. At 0.6 mg/kg/day, brain ChE was inhibited 60-62% in PND21 pups, 31% in GD20 dams, and 6-13% in adults; RBC ChE was inhibited 85-86% in PND21 pups, 58% in GD20 dams, and 40-58% in adults; and plasma ChE was inhibited 56-61% in PND21 pups, 29% in GD20 dams, and 28-34% in adults.

c. Summary of Differential Sensitivity

Table 1 shows results of ChE measurements performed in acute and repeat dosing studies with OP pesticides using neonatal, juvenile, or adult rats. The information provided in Table 1 is confined to data that could be used to estimate the relative sensitivities of different age groups based on the amount of ChE inhibition reported following treatment with an OP pesticide. Estimates of relative sensitivities (4th column) were derived by either: (1) the ratio of the response *(i.e.*, percent ChE inhibition) for adults: pups at the same dose of chemical, or (2) the ratio of doses in adults: pups that induce a comparable amount of ChE inhibition. The different approaches to estimating the relative sensitivities to a ChE-inhibiting chemical were necessary because of the differences in study designs and results among the studies evaluated. For example, some studies used single doses such as a proportion of an LD whereas other studies used multiple doses that allowed calculations of an ED_{50}. It should be noted that estimates of relative sensitivities are a function of the doses or percentages of ChE reported in studies and that, depending on dose-response characteristics of ChE inhibition among different age groups, actual sensitivities may be different at doses other than those used in Table 1.

[Since this report was prepared, preliminary $BMD_{10's}$ were derived for the dose-response ChE data from repeated dosing studies on pups and adults in RBC and brain for malathion (Fulcher 2001), methyl parathion (Beyrouty 2002)and chlorpyrifos (Zheng *et al.*, 2000). These data were modeled using the EPA Benchmark Dose Software version 1.3.1 - Hill model (available at website: http://cfpub.epa.gov/ncea/cfm/bmds.cfm?ActType=default). The modeling confirmed

that there was less than two-fold difference in response between adults and pups following repeated dosing with chlorypyrifos. For malathion, a difference between adults and pups up to approximately 3-fold was found for RBC ChE inhibition based on the BMD_{10s}. For brain ChE , there was 16% inhibition in pups at the highest dose tested (150 mg/kg/day) but no inhibition in adults. Thus, relative sensitivity could be determined because of the lack of comparable dose response data in pups and adults. Although Table 1 reports a differential in brain ChE inhibition for methyl parathion up to 3.-fold (comparing percent inhibition), modeling the Beyrouty data showed differences up to approximately 4-fold based on BMD_{10s}.]

Table 1. Summary of Sensitivity to ChE Inhibition in Neonatal or Juvenile Rats Treated with Organophosphorus Pesticides

Pesticide & Reference	Treatment Groups; Doses (mg/kg/day); Route of Administration	Results	Relative Sensitivity (Fold Difference)
Chlorpyrifos			
(Pope, 2001a)	Neonates, juveniles, and adults 7.5 neonates, 23.5 juveniles, 68 adults single gavage dose	**Neonates:** 70% ChEI in cortex at 7.5 mg/kg **Juveniles:** 65% ChEI in cortex at 23.5 mg/kg **Adults:** 60% ChEI in cortex at 68 mg/kg	**ACUTE** **Juveniles/neonates:** 23.5 mg/kg/ 7.5 mg/kg = 3.1 **Adults/neonates:** 68 mg/kg/ 7.5 mg/kg = 9.1
Moser *et al.*, (1998)	Adults and PND17 pups 20 mg/kg single gavage oral doses	**Pups Brain ChEI:** 89% (♂) and 91% (♀) **Adults Brain ChEI:** 39% (♂) and 66% (♀) inhibition in adults	**ACUTE** **Pups/Adults:** 89 % ChEI/39% ChEI=2.3; 91% ChEI/36% ChEI=2.3
(Zheng *et al.*, 2000)	Repeated gavage doses of 0.15, 0.45, 0.75, 1.50, 4.50, 7.50, or 15.0	**Pups:** ED_{50} for ChEI in cortex, 2.2 mg/kg/day **Adults:** ED_{50} for ChEI in cortex, 3.3 mg/kg/day **Pups:** 54.9% RBC ChEI, 1.5 mg/kg/day **Adult:** 91% RBC ChEI, 1.5 mg/kg/day	**REPEATED** **Adults/pups:** 3.3 mg/kg/day/2.2 mg/kg/day = 1.5 (no difference) **Adult/pups:** 54.9% ChEI/91% ChEI = 0.6 (no difference)
Diazinon			
(Padilla *et al.*, 2002)	Adults and PND17 pups single gavage dose of 75	**Pups:** 75% brain ChEI **Adults:** 35% brain ChEI	**ACUTE** **Pups/Adults:** 75% ChEI/35%ChEI = 2.1
Dimethoate			
(Myers, 2001; MRID 45529702, unpublished)	Adults and PND11 pups single gavage doses of 0.1, 0.5, or 3.0	**Pups:** 18% brain ChEI at 3.0 mg/kg; 26% RBC ChEI at 3.0 mg/kg **Adults:** 14% brain ChEI at 3.0 mg/kg; 27% RBC ChEI at 3.0 mg/kg	**ACUTE** **Pups/Adults:** At 3 mg/kg 18% ChEI/14%ChEI=1.3 26% ChEI/27% ChEI=1 (no difference)
	Adults and PND11-21 repeated gavage doses of 0.1, 0.5, or 3.0	**Pups:** 45% brain ChEI at 3.0 mg/kg/day; 13% ChEI at 0.5 mg/kg/day; 65% RBC ChEI at 3.0 mg/kg/day; no RBC ChEI at 0.5 mg/kg/day **Adults:** 60% brain ChEI at 3.0 mg/kg/day; 13% brain ChEI at 0.5 mg/kg/day; 63% RBC ChEI at 3.0 mg/kg/day; no RBC ChEI at 0.5 mg/kg/day	**REPEATED** **Pups/Adults:** At 3 mg/kg/day 45% ChEI/60% ChEI=0.8 (no difference) At 0.5 mg/kg/day 13% ChEI/13% ChEI=1 (no difference) At 3 mg/kg/day 65% ChEI/63% ChEI=1 (no difference)

Table 1. Summary of Sensitivity to ChE Inhibition in Neonatal or Juvenile Rats Treated with Organophosphorus Pesticides

Pesticide & Reference	Treatment Groups; Doses (mg/kg/day); Route of Administration	Results	Relative Sensitivity (Fold Difference)
Malathion			
Fulcher, 2001, MRID 45566201, unpublished)	Adults and PND11 pups single gavage doses of 5, 50, 150, or 450	**Pups:** 84% brain ChEI at 450 mg/kg; 48% brain ChEI at 150 mg/kg; 72% RBC ChEI at 450 mg/kg; 55% RBC ChEI at 150 mg/kg **Adults:** No brain ChEI at 150 or 450 mg/kg; 25% RBC ChEI at 450 mg/kg; no RBC ChEI at 150 mg/kg	**ACUTE** **Pups/Adults:** 84% brain ChEI/no brain ChEI at 450 mg/kg; fold difference uncertain **Pups/Adults:** 72% RBC ChEI/25% ChEI at 450 mg/kg = 2.9
	Adults and PND11-21 pups repeated gavage doses of 5, 50, or 150	**Pups:** 16% brain ChEI at 150 mg/kg/day; no brain ChEI at 50 mg/kg/day; 68% RBC ChEI at 150 mg/kg/day; 39% RBC at 50 mg/kg/day 17% RBC ChEI at 5 mg/kg/day **Adults:** No brain ChEI and 51% RBC ChEI at 150 mg/kg/day; 20% RBC ChEI at 50 mg/kg/day	**REPEATED** **Pups/Adults:** 16% brain CHEI/no CHEI at 150 mg/kg/day and no brain at 50 mg/kg/day in pups or adults; fold difference uncertain **Pups/Adults:** 68% RBC ChEI/51% RBC ChEI at 150 mg/kg/day =1.3; 39% RBC ChEI/20% RBC ChEI at 50 mg/kg/day =2.0
Methamidophos			
(Moser, 1999)	Adults and PND17 pups single gavage dose of 1, 4, or 8	**Pups:** ED_{50} for brain ChEI 3.0 mg/kg; ED_{50} for blood ChEI 2.3 mg/kg **Adults:** ED_{50} for brain ChEI 3.0 mg/kg; ED_{50} for blood ChEI 2.0 mg/kg	**ACUTE** **Pups/Adults:** 3 mg/kg/3 mg/kg=1 (no difference); 2.3 mg/kg/2 mg/kg=1.2 (no difference)
Methyl Parathion			
(Pope, 2001a)	Neonates, juveniles, and adults treated with a single gavage dose neonates 1.0, juveniles 2.05, adults 7.3	**Neonates:** 60% ChEI in cortex at 1.0 mg/kg **Juveniles:** 60% ChEI in cortex at 2.05 mg/kg **Adults:** 70% ChEI in cortex at 7.3 mg/kg	**ACUTE** **Juvenile/neonate:** 2.05 mg/kg/ 1.0 mg/kg = 2.05 **Adult/neonate:** 7.3 mg/kg/1.0 mg/kg = 7.3
(Beyrouty, 2002a; MRID 45656501, unpublished)	Adults and PND11 pups single gavage doses of 0.03, 0.11, 0.3, or 1.0 pups; 0.03, 0.11, 0.3, or 0.6 adults	**Pups:** 18% brain ChEI at 0.3 mg/kg; 31% RBC ChEI at 0.3 mg/kg **Adults:** No brain or RBC ChEI at 0.3 mg/kg	**ACUTE** **Pups/Adults:** 18% brain ChEI/no brain ChEI at 0.3 mg/kg; fold difference uncertain 31% RBC ChEI/no RBC ChEI at 0.3 mg/kg; fold difference uncertain
	Adults and PND11-21 pups repeated gavage doses of 0.03, 0.11, 0.3, or 0.6	**Pups:** 62% brain ChEI at 0.6 mg/kg/day; 29% brain ChEI at 0.3 mg/kg/day; 86% RBC ChEI at 0.6 mg/kg/day; 65% RBC ChEI at 0.3 mg/kg/day **Adults:** 31% brain ChEI at 0.6 mg/kg/day; 9% brain ChEI at 0.3 mg/kg/day; 58% RBC ChEI at 0.6 mg/kg/day; 35% RBC ChEI at 0.3 mg/kg/day	**REPEATED** **Pups/Adults:** 62% brain ChEI/31% brain ChEI at 0.6 = 2.0 29% brain ChEI/9% brain ChEI at 0.3 =3.2; 86% RBC ChEI /58% RBC ChEI at 0.6=1.5; 65% RBC ChEI/35% RBC ChEI at 0.3 =1.9

3. Recovery from ChE Inhibition in Young Rats Treated with Organophosphorus Pesticides

Studies that included analyses of recovery from ChE inhibition in young rats have been performed on chlorpyrifos, methamidiphos, methyl parathion, and parathion.

PND17 pups were reported to recover from ChE inhibition in one week after cessation of dosing with a single maximum tolerated dose (MTD) of **chlorpyrifos** of 15 mg/kg compared with a greater than two-week recovery period in adults administered a MTD dose of 80 mg/kg/day (Moser and Padilla, 1998). Pope *et al.* (1991) also reported a faster recovery in ChE activity in neonates compared to adults when treated with a MTD of chlorpyrifos. Adults treated with an acute, subcutaneous, dose of 279 mg/kg chlorpyrifos showed about a 90% inhibition of brain ChE activity seven days after dosing compared to approximately 40% inhibition in the brains of seven-day-old neonates treated with 45 mg/kg chlorpyrifos.

One day following oral treatment every other day with three doses of 3 mg/kg/day and eight doses of 6 mg/kg/day from PND1-21, brain ChE activity was inhibited by 57% (Tang *et al.*, 1999). Following a 19-day recovery period, brain ChE activity (about 20% inhibition relative to controls) was still depressed. Thus, although ChE levels in juvenile rats return to control levels after an acute treatment with chlorpyrifos, repeated treatments can lead to prolonged ChE inhibition.

PND17 and adult rats orally administered 8 mg/kg **methamidiphos** each showed about 80-85% brain ChE inhibition 1.5 hours after dosing (Moser, 1999). Twenty-four hours after dosing, more recovery was noted in the pups than adults (30-35% brain ChEI in pups versus 55% in adults). At 72 hours post dosing, ChE activity in pups had returned to normal but there was still brain ChE inhibition in adults (10-15%)

Neonatal (seven-day-old) pups and adults were found to have similar brain ChE activities (about 20% activity compared to controls) when administered a MTD of **methyl parathion** (7.8 mg/kg: neonates; 18 mg/kg: adults) but a more rapid recovery was reported for the neonates (Pope *et al.*, 1991). By seven days after treatment, neonatal ChE activity was almost completely recovered (about 90%) whereas brain ChE activity in adults was about 50% relative to controls.

Repeated treatments with methyl parathion of PND7 or 14 neonates and adults showed more inhibition initially but a faster recovery in the young rats (Liu *et al.*, 1999). On day 8, one day after seven days of subcutaneous treatment of neonates and adults, inhibition in the cortex of neonates administered 1.5 mg/kg/day was 73% (neonate) and 32% (adults). At a dose of 3.0 mg/kg/day, more inhibition was found in striatal than cortex tissues: Striatal inhibition at that dose was reported as 86% (neonate) and 64% (adult) one day following seven days treatment; seven days after cessation of dosing, brain ChE inhibition was about 45% in both age groups, indicating that more recovery had occurred in neonates (41%) than in adults (only 19%).

Liu *et al.* (1999) also investigated the effects on ChE inhibition in neonatal rats and adults following administration of MTD doses of **parathion**. As with methyl parathion, maximum brain ChE inhibition was similar (>85% on the day of dosing in neonates and 90% in adults four days after dosing), but recovery in the neonates was more rapid. Seven days after cessation of dosing, brain ChE activity had essentially returned to normal in neonates but brain ChE was inhibited by 80% in the adults.

C. Mechanisms Underlying the Differential Age-Related Sensitivity For ChE Inhibition

Age-related differences in sensitivity to pesticides can occur for a number of reasons (Pope, 2001b). Exposures to pesticides are age-related (discussed in Section D) where children may be more exposed than adults based on their diet and behaviors. Toxicodynamic and toxicokinetic differences may also contribute to the young being at a different risk to pesticide exposure. As summarized below, there are several reports in the literature investigating the basis underlying the differential sensitivity found for certain OP pesticides.

Toxicodynamic Considerations: There may be different mechanisms underlying age-related sensitivity to ChE inhibition for different OP pesticides. The exact mechanisms are not clearly understood. For obvious reasons, no data available in humans. There are studies, however, in laboratory animals that provide some information. Intrinsic differences in neuronal AChE (*i.e.*, differential inhibition of the target enzyme itself) do 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). Another toxicodynamic factor, the ability to restore function following exposure, does not appear to be the basis for age-related sensitivity to the OP pesticides because more rapid recovery of AChE activity in younger animals is found compared to adults (Chakraborti *et al.,* 1993; Moser, 1999; Pope *et al.*, 1991; Pope and Liu, 1997).

Other toxicodynamic differences that could affect age-related sensitivity to AChE inhibition concern the regulation of acetylcholine receptor number as well as acetylcholine release. Inhibition of ChE activity in the nervous system results in the accumulation of acetylcholine in the synapses causing hyperstimulation of the cholinergic receptors on postsynaptic cells. It is this hyperstimulation that leads to cholinergic toxicity. This hyperactivity may also lead to a decrease in the number of muscarinic receptors (*i.e.,* down-regulation). As a measure of toxicodynamic response to OP dosing, some studies have compared the degree of muscarinic down-regulation in adult and young rats. In a study of repeated, subcutaneous dosing with methyl parathion or chlorpyrifos, Liu *et al.* (1999) found that muscarinic receptor number was markedly reduced in pups compared to adult rats following repeated dosing with methyl parathion, suggesting age-dependent differences in mucarinic receptor adaptation. Interestingly, the chlorpyrifos exposure also produced more receptor down-regulation in the pup as compared to the adult, but the effect was not as pronounced as the methyl parathion effects. Moreover, using a different route, the same group showed that repeated oral dosing with chlorpyrifos caused equal down-regulation of muscarinic receptors in neonatal and adult brain (Zheng *et al.,* 2000). The effect on receptor down-regulation appears to be compound-specific, and possibly, route-specific. In the normal cholinergic synapse, it is known that feedback inhibition of acetylcholine release occurs through activation of the muscarinic acetylcholine receptors located on the presynaptic nerve terminals (see Pope, 2001b). Activation of the muscarinic acetylcholine receptors would decrease further acetylcholine release, thereby reducing the excessive stimulation of the postsynaptic receptors following AChE inhibition. A limited ability or adaptability of this presynaptic regulatory process in the young could lead to increased sensitivity to OP pesticides. There are only a few reports exploring age-related differences in muscarinic presynaptic acetylcholine receptor activity: evoked acetylcholine release was lower in brain tissues from newborn animals and aged animals compared to rats aged one to six months (Pedata *et al.,* 1983; Meyer and Crews, 1984). There is no information on the receptor response (either total muscarinic receptor number or feedback inhibition of acetylcholine release) in the developing human brain.

Toxicokinetic Considerations: Toxicokinetic differences among age groups can contribute to age-related differences in response, with the interplay of metabolic activation and detoxification processes being an important major factor, particularly in the first few months after birth (*e.g.,* see Ginsberg *et al.*, 2002). It appears from the literature that toxicokinetic differences play an important role in the differential sensitivity of the young to ChE inhibition following treatment with OP pesticides (*e.g.,* Brodeur and DuBois, 1963; Benke and Murphy, 1975; Scheidt *et al.,*1987, reviewed in Pope, 2001b). In addition to inhibiting AChE, OP pesticides also interact with other esterases, *i.e.*, carboxylesterases and/or A-esterases, an by doing so become inactivated or detoxified. A-esterases (*e.g.*, chlorpyrifos oxonase, paraoxonase, or PON1) detoxify some OP pesticides by hydrolysis, whereas some OPs bind to carboxylesterases, a reaction which effectively lessens the amount of pesticide available for inhibiting AChE. Many investigators have noted the decreased capability of the young animal to detoxify OP pesticides by A-esterase or carboxylesterase esterases compared to adults (Mortensen *et al.,* 1996; Atterberry

et al., 1997; Costa *et al.*, 1990; Padilla *et al.*, 2000; Padilla *et al.,* 2002; Karanth and Pope, 2000).

Laboratory Animal Literature: The importance of A-esterase protection against the toxic effects of the anticholinesterase activity of OP pesticides has been demonstrated in several studies in which exogenous administration of A-esterase can lessen OP toxicity in rodents (Costa *et al.*, 1990; Li *et al.*, 1993; Main, 1956). Studies with an A-esterase knockout mouse reinforced the important role that A-esterases play in the detoxication of OP pesticides: knockout mice were much more sensitive to chlorpyrifos oxon or diazoxon (the active metabolites of chlorpyrifos or diazinon, respectively) than their wildtype litter mates (reviewed in Furlong *et al.*, 2000). 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). 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), but there are sparse data on the role of carboxylesterase activity mediating age-related toxicity to OP pesticides. Fetal rats possess very little carboxylesterase activity (Lassiter *et al.*, 1998) with increasing 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).

The temporal pattern of A-esterase activity (and carboxylesterases) correlates reasonably well with studies on OP sensitivity (see summary in Table 2). 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). For example, Padilla *et al.* (2002) and Karanth and Pope (2000) have correlated age-related sensitivity with the maturational profiles of these esterases. Using an *in vitro* assay, Padilla *et al.* (2000) showed that methamidophos, a pesticide which is not more toxic to the young rat, is not detoxified by A-esterases or carboxylesterases. These observations have been extended in a recent abstract to other OP pesticides using this *in vitro* model which measures the detoxification potential via these esterases in various tissues (*e.g.*, liver, plasma) (Padilla *et al.*, 2002). It was reported that the differential sensitivities of paraoxon (the active metabolite of parathion), malaoxon (the active metabolite of malathion), and diazoxon (the active metabolite of diazinon) were also correlated with the less efficacious detoxification by these esterases in young animals (Table 2). Karanth and Pope (2000) noted that the lower levels of esterases in neonatal and juvenile rats correlated with the increased *in vivo* sensitivity to ChE inhibition found for chlorpyrifos and parathion.

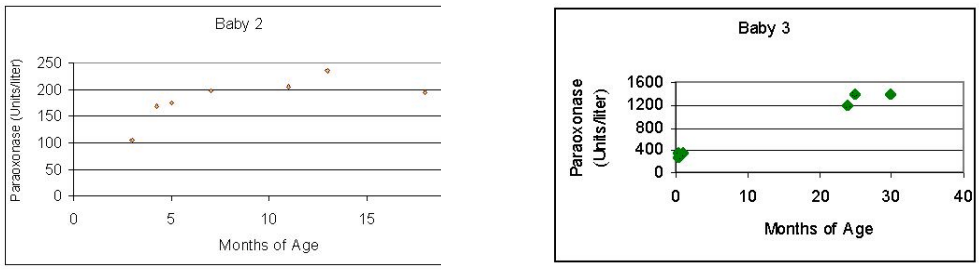
Table 2. Summary of General Results for Age-Related Detoxification and Sensitivity in Rat Studies

Pesticide	Hydrolyzed by A-Esterases?	Bind to Carboxyl-esterases ?	Age-Related Detoxification?	More Sensitive to Young?	Reference
Chlorpyrifos	Yes	Yes	Yes	Yes (acute dose of PND No (repeated dosing of)	Karanth and Pope, 2000;Padilla *et al.*, 2002
Diazinon	Yes	Not Much	Yes	Yes	Padilla *et al.,* 2002
Dimethoate	Not tested	Not tested	Not tested	No	Meyers, 2001
Malathion	No	Hydrolyze d	Yes	Yes	Fulcher, 2001; Padilla *et al.,* 2002
Methamidophos	No	No	Not tested	No	Moser, 1999; Padilla *et al.*, 2000
Methyl Parathion	No	Yes (limited)	Yes	Yes	Pope, 2002a; Chambers and Carr, 1993
Parathion (not included in cumulative assessment)	No	Yes	Yes	Yes	Karanth and Pope, 2000; Padilla *et al.,* 2002

Human Literature: There are only a few studies in the older 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, 1963; Mueller *et al.,* 1983; Ecobichon and Stephens, 1972). After birth, there is a steady increase of this activity during the first six months to about one year (Augustinsson and Barr, 1963). In a related study of the age-dependence of total serum arylesterase activity (of which a large component is A-esterase activity), adult levels were achieved by two years-of-age (Burlina *et al.*, 1977). Although serum A-esterases are reported to achieve adult levels around six months to one year-of-age, there is uncertainty surrounding those values for the one-year-old due to the variability in the rate of maturation expected as these enzyme systems mature at different rates in a cross-section of one-year-old children. Suggestive evidence of this is the large degree of variability seen in the six-month and one-year age groups in the limited serum esterase data available for children (Augustinsson and Barr, 1983). This source of variability (maturational rate) is unique to children and is in addition to the host of factors that contribute to interindividual variability in the rest of the population and normally considered in noncancer risk assessments. Given the small number of children studied for this parameter, population distributions that reflect the central tendency and lower percentile value for A-esterase function in one-year-olds relative to adults cannot be discerned from the data (for example see Ecobichon and Stephens, 1972; Figure 1). Moreover, these studies have only examined a few children, and given the high interindividual variability, it is very difficult to discern with confidence the maturation

profile for serum A-esterases in young children. In ongoing studies in C. Furlong's laboratory, the same child is being evaluated for the appearance of serum A-esterase over time (*i.e.*, so that the high natural variation does not obscure developmental patterns) to better define the developmental profiles for serum A-esterases (See Figure 1 below). Preliminary results indicate that children reach adult levels of A-esterases around 12 to 15 months-of-age. Note that this age of maturation corresponds reasonably well with the maturation of human serum arylesterases mentioned above (Burlina *et al.*, 1977). It should also be pointed out that there is no information on the maturational profile of A-esterase in the human liver (an organ very important for detoxification), and there appears to be no information about the maturational profile of carboxylesterases in humans.

Figure 1. Maturation Profile of Serum A-Esterase (Paraoxonase) Appearance in Infants and Children (Costa *et al.*, 2002).



Any anticholinesterase pesticide that is a substrate for A-esterase, the lower A-esterase levels in the blood of very young would result in more inhibitor available to reach target neuronal tissues. It should be noted that in addition to age-dependent differences in A-esterase activity, a human and animal genetic polymorphism has been well established (*e.g.*, Mackness *et al.*, 1998). Differences in observed rates of hydrolysis of paraoxon between individuals can vary by at least 20-fold (Furlong *et al.*, 2000). This large difference in A-esterase activity does not necessarily translate into equivalently large differential sensitivities to OP pesticides. There is also some recent evidence in the literature that low A-esterase activity may predispose adult humans to a greater toxic response (Haley *et al.*, 1999; Cherry *et al.*, 2002) to nerve agents and/or pesticides.

Not only is limited detoxification a factor in increased sensitivity of the young, but another potential factor is the age-dependent ability to activate OP pesticides via oxidation by cytochrome P450s to their oxon form (*i.e.*, the active anticholinesterase metabolite). For example, oxidation by CYP3A4 plays a key role in the oxidation of

OP pesticides in humans (Butler and Murray, 1997). Ginsberg *et al.* (2002) using the children's pharmacokinetic data from the therapeutic drug literature showed that compared to adults, oxidation by CYP3A4 tends to be more active in children beginning as early as two to six months-of-age with this difference lasting until at least two years-of-age. While this may increase concern for greater oxidative bioactivation in the young, the CYP-mediated oxidative dearylation pathway, which may also be more active at these ages, is involved in the detoxification of these pesticides. Therefore, it is important to compare the maturation profiles for these two CYP pathways. Based on data from rat liver microsomes (Ma and Chambers, 1994) and as modeled by Timchalk *et al.* (2002) for humans and rats, the activation step is 2.5-fold faster (based upon Vmax/Km ratios) and importantly, the activation step has a 8.4-fold lower Km than the dearylation step. The significance of this is that at relatively low, environmental exposures, OP molecules reaching the liver may be much more likely to be oxidized by the activation pathway than detoxified by the dearylation pathway. This evidence supports the potential concern that greater oxidative capacity in one- to two-year-olds may lead to more OP activation than seen in adults. The enhanced ability of the young to bioactivate OP pesticides to their oxon form, however, has not been correlated with an increased sensitivity to ChE inhibition. Nonetheless, when coupled with the potential limited ability of young children to detoxify these pesticides via the A-esterase and carboxylesterase pathways, this produces a source of uncertainty in the pesticide risk assessment for children.

D. Hazard Characterization Summary

There have been reports of signs and symptoms associated with cholinergic toxicity following high exposures to OP pesticides of adults and of young children. Common signs and symptoms of cholinergic toxicity in humans range from changes in heart rate and blood pressure, miosis, diarrhea, headaches, nausea, muscle weakness to unconsciousness, convulsions, and death. Not only can cholinergic toxicity occur in children following exposure to OP pesticides, but emerging investigations have raised concern about the effects of antiChE activity on neurodevelopment which may be a sensitive process susceptible to adverse perturbations.

As discussed in Section A, there is evidence that ChE and acetylcholine act as important neuromodulators in the developing brain. Because neurogenesis is not limited to the intrauterine period and may continue throughout childhood, all stages of brain development are considered to be potentially susceptible to disruption by ChE inhibition. During the first few years of life, brain development is a tightly orchestrated process of migration and “pruning,” which is under the influence of neuromodulators (ChE, acetylcholine, and other neurotransmitters), genetic controls, and the experiences of the child. Although OP pesticides may influence the migration of cells and the connectivity of the central nervous system (CNS) and result in consequences that could last into adulthood, it is not known how much of a perturbation (*i.e.*, degree of ChE inhibition) is needed, or how long this perturbation must be sustained, to disrupt normal development. The majority of OP pesticides

included in the cumulative risk assessment have not been evaluated for neurodevelopmental effects (*e.g.*, functional, behavioral, or neuropathogical effects) or for ChE activity in immature animals.

In light of this uncertainty, it should be assumed that small perturbations resulting from either a single exposure or repeated exposure could potentially disrupt neurodevelopment. Therefore, it is important to insure that the adult brain ChE endpoints used in the cumulative risk assessment for OP pesticides are adequately protective of the young. Thus, a key issue in this assessment is whether ChE inhibition in the young will be caused at lower doses of these pesticides compared to adults or whether the young will show a higher level of ChE inhibition at comparable doses. It is the integration of the chemical-specific information along with the basic biological understanding of sensitivity and susceptibility that informs the need for the application of additional safety or uncertainty factors in the cumulative risk assessment to protect fetuses, infants, and children.

Because in humans, the process of brain development begins during gestation and continues postnatally through adolescence, it is important to identify the developmental windows of age-dependent sensitivity to ChE inhibition. In laboratory animals, ChE inhibition can be found to occur in all developmental stages of the young (*i.e.*, in fetal, neonatal, juvenile, and young adult rat tissues). In general, oral dosing of pregnant rats with OPs causes ChE inhibition in the fetus and/or neonate, but fetuses/neonates that are exposed *in utero* (and via early lactation) generally do not exhibit more ChE inhibition than is found in maternal tissues. These studies need to be interpreted with caution with respect to comparative sensitivity because the absorbed dose to the dam and fetus is typically not known. Also, the fetal rat appears to be less affected from repeated exposures to OP pesticides presumably because of the rapid recovery and resynthesis of the AChE in fetal tissue compared to the dam, making it difficult to compare relative responses in the fetus versus dam. It should be noted that rat fetal tissues and the placenta are deficient in key detoxification systems, including A-esterases and carboxylesterases. Overall, there is limited pharmacokinetic information available in fetal versus maternal tissues for OP pesticides.

Continued treatment following birth is important to ensure that critical periods of sensitivity are evaluated. Direct dosing of the postnatal rat may be necessary, however, because of the possibility of limited exposure through the milk via lactational transfer of OP pesticides. Although direct dosing of the pups (typically via oral gavage) maximizes and allows for quantification of exposure to the pups, it does not necessarily mimic the dietary intake exposure patterns in children. Furthermore, certain stages of brain development in the early postnatal rat are equivalent to the third trimester human fetus, and thus direct dosing of very young postnatal rats would not represent the pharmacokinetics of the chemical in the mother. Nonetheless, direct dosing experiments do provide a better basis to determine the comparative sensitivity of the pups and adult animals. Some direct dosing studies of postnatal rats are available on OP pesticides; however, these few studies have shown that acute postnatal exposures via direct dosing to young rats results in an

increased sensitivity to ChE inhibition for certain OP pesticides (*e.g.*, malathion, methyl parathion, chlorpyrifos, diazinon), but not all (*e.g.*, methamidophos, dimethoate and by extension, its metabolite omethoate).

Age-dependent sensitivity to ChE inhibition by OP pesticides can sometimes also be found following repeated dosing studies in laboratory animals. An important issue with repeated dosing is the more rapid recovery (synthesis of new ChE enzyme) in postnatal (and fetal) rat tissues. In most repeated dosing studies comparing the responses of adults to postnatal animals dosed at the same frequency, this faster recovery in the young animals may result in less inhibition as compared to the adults, which is interpreted by some as lower sensitivity of the young. The results of such studies are critically dependent on the time interval between the doses and also the time (in relation to the last dose) at which the ChE inhibition is sampled in both age groups. As acute studies have shown, age-related sensitivity differences in rodents depend on the age at dosing, since the detoxification pathways are rapidly maturing. Therefore, in repeated dose studies, the fact that the animals are probably becoming less sensitive over time by virtue of this changing toxicokinetic pattern is an additional confounding factor. For all these reasons, a smaller differential for ChE inhibition has often been found between the pups and adults following repeated dosing when compared to acute exposure.

Although age-dependent sensitivity is found in some animal experiments, a key question is whether this sensitivity will occur in children. Children may respond to toxicity at lower doses than adults because infants and very young children may be less able than adults to metabolize and excrete toxic substances (Ginsberg *et al.*, 2002). Animal studies have shown a correlation of age-dependent sensitivity to certain OP pesticides with the developmental profiles of the A-esterases and/or carboxylesterases (enzymes that detoxify OP pesticides). As described in Section C, based on limited data, young children may have lower levels of these detoxification pathways. The most highly exposed age group in the OP cumulative risk assessment was identified as the one- to two-year-olds. Although after birth there is a steady increase of A-esterase activity during the first six months to one year, these maturation profiles may vary among children (due to interindividual variability) and may vary among different tissues. Maturation profiles are not available for the carboxyesterases, and the developmental profile for either A-esterases or carboxylesterase has not been delineated in liver (a major detoxication organ). Furthermore, young children may also have an increased ability to activate OP pesticides to the oxon form as compared to the adult. Therefore, given the uncertainty surrounding the maturation profiles of young children for A-esterases and carboxylesterase, their potential to be more active than adults at bioactivating OP pesticides to their oxon form, as well as their rapidly developing nervous system, infants and very young children (including children in the one- to two-year age group) would potentially be vulnerable to chemical interference due to OP pesticide exposure.

Because some OP pesticides do show age-dependent sensitivity, and there are missing ChE data in young animals for many of the OP pesticides in this cumulative risk assessment, there is a degree of uncertainty regarding the estimation of risk. Under the children's safety factor provision a default safety factor of 10X is required to address this database deficiency unless there are reliable data to support a conclusion that a different safety factor would be safe for infants and children. As the following discussion indicates, OPP has concluded that reliable data do exist to support use of a database uncertainty factor to address this data deficiency. To determine whether a database uncertainty factor could protect infants and children, the degree of difference between the doses needed to cause a certain level of ChE inhibition between the young and adult was evaluated. As shown in Table 1, the differential between adults and immature animals following repeated dosing (typically 11 consecutive days) is at most approximately threefold. A single acute dose is found to cause differences ranging from about twofold up to approximately ninefold.

The relative sensitivities of immature animals found in repeated dosing studies are considered more appropriate than the results of the acute dosing studies for the cumulative risk assessment of OP pesticides for several reasons. Acute dosing studies were done with PND11 pups, which are more like the human newborn with limited detoxification ability. Repeated dosing studies of OP pesticides usually started treatment at PND11 and continued to PND21. As the immature animal ages, it rapidly reaches adult levels of A-esterases around PND21. Thus, evaluation of ChE activity in repeated dosing studies more closely mimics the maturation of A-esterase activity in children around one year-of-age when children are reaching adult levels of A-esterases. Thus, the use of repeated dosing studies better approximates the maturation profile of the age group that is significantly exposed to OP pesticides in the cumulative risk assessment. Children generally do not begin to consume fresh (uncooked) fruits and vegetables until after six months-of-age or more. The highly exposed group in the cumulative risk assessment is the one- and two-year-olds, not the infants. Repeated dosing studies were also used to determine relative sensitivity because people are exposed every day to an OP pesticide through food, and thus an animal study using repeat exposures is considered appropriate. Also, following exposure to an OP, regeneration of ChEs to preexposure levels does not occur for days or weeks, making the exposed individual potentially more vulnerable to subsequent exposures during that period.

Repeated dosing studies are now available on six of the 22 OPs in the cumulative risk assessment. For three of these OP pesticides, the repeated dosing studies showed no increased sensitivity in the young, whereas increased sensitivity was seen in the other three. The differential sensitivity between adult and immature animals ranged from 1X (*i.e.*, no differential) up to a 3X difference. These studies are considered to provide a reasonable basis on which to establish the size of a database uncertainty factor for the following reasons. Although these six OP pesticides do not represent every structural and pharmacokinetic characteristic of the large class of OP pesticides included in the cumulative risk assessment, they are nonetheless a reasonable subset of different structural and pharmacokinetic characteristics. For example, methamidophos is a phosphoramidothioate of small

molecular weight with no ring structure, does not require metabolic activation to generate an oxon form, and is not detoxified by esterases. On the other hand, methyl parathion is a phosphorothioate of larger molecular weight with a ring structure, hepatically bioactivated to its oxon form, and detoxified by esterases. In addition to the observed differential between adult and young animals following repeated dosing, it must also be kept in mind that the differential between the adult and young animal decreases as the animal ages and reaches adult levels of the detoxification enzymes. For these reasons, there are sufficient data to conclude that a 3X database uncertainty factor should be applied, and that the 3X UF_{Db} should be sufficient to account for potential age-dependent sensitivity to ChE inhibition. It should be noted that the application of a 3X UF_{Db} is in addition to the application of the customary intra- and interspecies uncertainty factors, which takes into account variability among the human population.

The question remains as to how such a database uncertainty factor should be incorporated into the cumulative risk assessment. In the cumulative risk assessment process, uncertainty or safety factors can be either applied to estimates of individual member's toxic potencies (*i.e.*, relative potency factors or RPFs) or applied as a group factor on the index chemical's point of departure.[3] Because age-dependent sensitivity to ChE inhibition is not common to all OP pesticides, application of a database uncertainty factor would be more appropriately applied as chemical-specific adjustments to the RPFs to account for ChE inhibition potentially occurring at lower doses in the young than in the adult or resulting in a more potent response at the same dose compared to the adult. These chemical-specific adjustments should be made on the RPFs for those OP pesticides that lack ChE data in the young. There are ChE data for a few OP pesticides that show age-dependent sensitivity. However, RPFs are based on a uniform measure of toxic potency using the same species, sex, endpoint, and age group from studies of comparable methodology. Given that there are too few data in young animals to determine RPFs for the OP Cumulative Assessment Group (CAG), the RPFs for those chemicals showing age-dependent sensitivity should also be adjusted to account for sensitive effects in the young. The RPFs of those OP pesticides that do not cause age-dependent sensitivity after brief periods of repeated exposure (dimethoate and by extension omethoate, methamidophos, chlorpyrifos) should not be adjusted.

[3]In the cumulative risk assessment, the RPF approach is used to determine the joint risk of the OP pesticides, which applies dose addition. The RPF approach uses an index chemical as the point of reference for standardizing the common toxicity of the chemical members of the (CAG). Relative potency factors (*i.e.*, the ratio of the toxic potency of a given chemical to that of the index chemical) are then used to convert exposures of all chemicals in the CAG into exposure equivalents of the index chemical.

In conclusion, the limited animal data on the relative sensitivity of young animals to cholinesterase inhibition (ChEI) caused by OP pesticides has raised uncertainty about the adequacy of the adult RPFs to be protective of the young and should be addressed by application of the traditional database uncertainty factor (UF_{Db}). Application of this UF_{Db} should be protective of potential age-dependent sensitivity to ChE inhibition and of potential adverse neurodevelopmental outcomes that are a result of the inhibition of ChE. Thus, there are reliable data to assign an additional factor (a database uncertainty factor of 3X) other than the default 10X additional safety factory. Further, because the database uncertainty factor addresses potential age-dependent sensitivity there is no a need to retain an additional special FQPA safety factor for potential pre- or postnatal toxicities associated with inhibition of ChE.

III. CUMULATIVE EXPOSURE ASSESSMENT

Another important consideration for the FQPA safety factor is the completeness of the exposure database. Whenever appropriate data are available, OPP estimates exposure using reliable empirical data on specific pesticides. In other cases, exposure estimates may be based on models and assumptions (which in themselves are based on other reliable empirical data). This section explains how the safety of the exposure estimates to infants and children were estimated.

EPA identified and included three exposure pathways for the OP Pesticide cumulative risk assessment: food, drinking water, and residential/nonoccupational. Each of these pathways was evaluated separately, and, in doing this step of the analysis, EPA determined which of the OP pesticides were appropriately included for a particular pathway. The cumulative assessment of potential exposure to OP pesticides in food includes: 22 OP pesticides that are currently registered in the U.S. or have import tolerances; residential or nonoccupational pesticide uses included 11 OP pesticides (Note: many residential uses have been canceled as a result of risk mitigation efforts or are not expected to result in any significant exposure); and 24 OP pesticides (as well as several toxic transformation products) were considered in the cumulative water exposure assessment. Calendex™ software was used to determine the distribution of exposures and resulting Margins-of-Exposure (MOEs) from OPs in foods, drinking water and from residential uses.

Up until this time, OPP has performed its risk assessments using several different age groups for children including nursing infants less than one year, non-nursing infants less than one year, children one to six years-old, children seven to 12 years-old, and children 13 to 19 years-old. Because of the availability of more extensive data on children's food consumption, EPA was able to subdivide the children's age group one through six years-of-age into two different age groups: children one through two years-old and children aged three through five years-old. EPA also made some other slight adjustments to the age breaks defining groups for older children. As explained below, EPA analyzed all of the different children's age groups, but did not analyze every age group for every scenario. The children's age groups that were analyzed for all of the exposure scenarios in the revised OP cumulative risk assessment were one through two years-of-age and three through five years-of-age. EPA selected these two age groups because in single chemical risk assessments (including for the individual OPs) they most frequently reflect the highest levels of exposure. Thus, a narrow range of ages were used to capture the finer details associated with major contributors to risk under the premise that they reflect the exposure scenarios most likely to be emphasized in risk management activities.

In addition, EPA produced exposure estimates for all of the other children's age groups (children less than one year, children six through 10 years and children 11 through 19 years) for the Florida region. Florida was selected because it appears to have the highest level of exposure from all sources of pesticides combined. As expected, the exposures estimated for children less than one year-old or six and older were consistently lower than the exposures estimated for one- to two- and three- to five-

year-old children. Based on this analysis, EPA concludes that, by focusing on two age groups of children (one- to two-year-olds and three- to five-year-olds), its risk assessment does not underestimate potential exposure to any age group of children.

A. Food Pathway

Exposure to foodborne pesticides is an important factor in evaluating the susceptibility of the young. Young children tend to eat more food in proportion to their body size and they tend to eat more frequently than adults. As discussed below, these characteristics are incorporated in the assessment of exposure to OP pesticides via food.

The food component of the cumulative risk assessment has been highly refined to reduce OPP's Tier 1 default assumptions (all foods contain residues at the maximum amount allowed under tolerance) to more realistic estimates of actual human exposure. It is based on residue monitoring data from U.S. Department of Agriculture (USDA) Pesticide Data Program (PDP), supplemented with information from the U.S. Food and Drug Administration (FDA) Surveillance Monitoring Programs and Total Diet Study. The PDP data provide a very reliable estimate of pesticide residues in the major children's foods. They also provide direct measures of co-occurrence of OP pesticide residues in the same sample, alleviating much of the uncertainty about co-occurrence in foods that are monitored in the program.

Another important aspect of the food exposure assessment is that it is based on actual consumption data from the USDA's Continuing Survey of Food Intakes by Individuals (CSFII). The CSFII provides a detailed representation of the food consumption patterns of the U.S. public across all age groups, during all times of the year, and across the 48 contiguous states. Additionally, OPP used a more recent CSFII in the OP cumulative assessment (the 1994-96 CSFII) that was supplemented in 1998 by the Supplemental Children's Survey. This 1998 survey focused on children from birth to nine years-old and greatly expanded (by several fold) the number of birth to four-year-old children in the survey database. OPP believes that the food consumption information used provides a very realistic estimate of potential risk concerns because it reflects the current eating habits of the U.S. public, including those of children. The use of the newer CSFII and the finer age breakouts should increase the accuracy and utility of the risk assessment overall by making it more descriptive of the anticipated exposures and risks for children.

A large percentage of the foods consumed in children's diet is addressed in this assessment. Only about 3% of the foods consumed by children still remained unaccounted for after using PDP and the FDA Total Diet Study and FDA monitoring data.

OPP is aware that some or all baby food manufacturers have adopted policies that restrict the use of OPs on fruits and vegetables that will be used in their products. As a result, children consuming commercially prepared baby food may not be exposed to OPs in their diet. OPP has investigated the impact of this assumption

for children one through two years-of-age, and for children less than one year-old. The residues in commercially prepared baby foods were assumed in the first case to be equivalent to those found in an adult diet. They were also set to zero to bound the lower limit and determine the extent of the impact on any risk assessment. Setting all residues in commercial baby food to zero had little impact on the magnitude of risk estimated for children one through two years-of-age. This observation is consistent with the very small amounts of baby food consumed by this age group. However, a substantial impact was observed for the age group of children less than one year-of-age because of the relatively large proportion of baby food in their diets. OPP believes that estimating exposure to pesticides from baby food as containing residues comparable to those in adult diets will not impact regulatory decision-making because the overall exposure to children less than one year-of-age is less than exposure to children one through two years-of-age.

Two exposure issues unique to children are not directly addressed in the current assessment. OP exposure from breast milk is not incorporated quantitatively. A review of the literature to identify any potential pesticide transfer from breast milk to children indicated no evidence that this pathway would represent a significant source of exposure (ILSI, 1998). However, further analysis has identified a study that demonstrated the presence of chlorpyrifos and chlorpyrifos-oxon in the milk of rats (Mattsson *et al.*, 1998, 2000). The results of studies generated by the regulated community in support of pesticide registration indicate no significant transfer of OPs to milk. OP pesticides are not found to transfer into cow's milk when cattle are fed a diet containing OPs. This finding is uniform across the entire class of OP pesticides. As a result, OPP believes that breast milk is not likely to be an important contributor of OPs to the diets of infants and children, especially at environmentally relevant levels of exposure. Baby formula is included in the current assessment with its consumption reflected in the FCID (Food Commodity Intake Database) translation of CSFII food consumption survey, and residue data available for all of its components.

OPP's dietary assessment also captures the metabolites of OPs that are known to occur at significant levels in food commodities (*e.g.*, omethoate–metabolite of dimethoate; methamidophos–metabolite of acephate; and, dichlorvos–metabolite of naled and trichlorfon). Although there is not extensive analytical data on other OP metabolites, there is adequate data (*e.g.*, from metabolism studies, FDA monitoring data) to indicate that the food assessment is not missing significant residues in food (such as for malaoxon– metabolite of malathion).

In summary, given the comprehensive data on potential exposure to OP pesticide residues through the food, OPP is confident that exposure to all age groups, including children via the food dietary pathway has been well characterized.

B. Drinking Water Pathway

Daily drinking water exposure estimates were generated using the simulation models PRZM and EXAMS (a description of the use of these models can be found http://www.epa.gov/oppefed1/models/water/index.htm). The use of these models allows estimation of concentrations of OP pesticides. OPP used these models to provide daily distributions of OP pesticide levels in water for incorporation into the probabilistic cumulative exposure assessment. Twelve regional water exposure assessments were conducted that were designed to represent exposures from typical OP usage conditions at one of the more vulnerable surface watersheds in the region. Each regional assessment focused on areas where combined OP pesticide exposure is likely to be among the highest within the region as a result of total OP usage and vulnerability of the drinking water sources. These methods have provided OP pesticide distributions that are, in many cases, reasonably comparable with available monitoring data in the same or nearby locations. There are too little data to quantify OP degradates that may result in drinking water. These metabolites, however, have been qualitatively assessed in the revised cumulative risk assessment by assuming complete oxon conversion with a 10-fold increase in toxicity compared to the parent compound: it was found that this assumption did not have an impact on the upper percentile distributions. In summary, OPP believes that the current cumulative assessment represents a reasonable, health protective estimate of likely exposure to OP pesticides from water to all age groups, including children.

C. Residential or Nonoccupational Exposure Pathway

The residential/nonoccupational exposure analysis includes the exposure from home lawn and garden treatments, pesticides used on golf courses, and applications made by governmental entities for the control of public health pests such as wide area mosquito sprays. The oral, dermal, and inhalation routes are considered. This analysis has incorporated activity patterns of children and the major sources of exposure to young children (*e.g.*, nondietary ingestion and hand-to-mouth behavior as established by video tapes of children). Furthermore, pet uses have been incorporated in the revised assessment. For the first time, the residential analysis used distributions of data and exposure elements instead of point values. In most cases, these data and exposure elements were chemical-specific. The analysis reflects all remaining residential uses of OP pesticides, consideration of both homeowner and professional applications, and postapplication exposures resulting from these applications. The analysis also employed the most recent survey data of residential uses and use information. Exposure due to activity in and around schools and parks is not addressed directly, because there does not appear to be any remaining OP pesticide uses for school structures and grounds. Nonetheless, the possibility of exposures encountered away from the home has been indirectly built into the assessment by conservatively extending the duration of residential exposure beyond the two hours spent on grass to 3.5 hours spent outdoors.

The calendar-based model (Calendex™) that was used in the preliminary OP

cumulative risk assessment allowed for the temporal aspects of the residential use of pesticides in twelve distinct geographic regions to be accounted for; these regions not only represent major crop growing areas and their influence on residues of OP pesticides in surface and ground water, but also present an opportunity to consider the unique climate patterns, pest patterns and potential socioeconomic patterns that influence residential pesticide use and expected exposure to OP insecticides. Furthermore, Calendex™ allows one to delineate the critical timing aspects of seasonal uses of OP insecticides that result in exposure, as well as to identify potential co-occurrences from multiple sources. Again, it cannot be emphasized too strongly that the exposure, monitoring, and residue studies that were used as input parameters in the modeling of residential/nonoccupational exposures represent the best available data on these pesticides (*i.e.*, the input parameters for residue levels and dissipation rates based on actual measurements).

D. Biological Monitoring Studies of Children

Biomarkers can serve as a useful measure of direct exposure aggregated over all sources and pathways by measuring integrated exposure from all routes. Biomarkers can be used to characterize the relative magnitude of exposure within population subgroups. In addition, biomonitoring can be used to verify predictions of exposure models.

Urinary biomarkers of OP pesticides and their metabolites have been used to characterize reference body burden levels for adult and children populations in the U.S. and Europe (Murphy *et al.,* 1983; Kutz *et al.*, 1992; Hill *et al.*,1995; Aprea *et al.*, 1996, 1999, 2000; Macintosh *et al.*, 1999; Fenske *et al.*, 2000; Quackenboss *et al.,* 2000; Adgate *et al.*, 2001; Heudorf and Angerer 2001; Krieger *et al.,* 2001). Most of this research has been designed to determine if children have higher exposures to OP pesticides than adults, and, if so, what are the differences in these exposures and what are the factors that influence these higher exposures.

Several researchers have conducted monitoring studies that have collected environmental and/or biological samples to assess the potential aggregate (inhalation, dermal, ingestion (dietary and indirect)) exposure to OPs by adults in their everyday environments. Hill *et al.* (1995) analyzed single spot urine samples from approximately 1000 adults (20-59 years-of-age) living in the U.S. to establish reference range concentrations for OP pesticide residues. Chlorpyrifos exposure was indicated by TCPY concentrations of 13 µg/L (95th percentile value) and 77 µg/L (maximum value observed). Macintosh *et al.* (1999) reported on the relationship between short-term and long-term average levels of OP biomarkers for 80 adults living in Maryland. First-morning void urine samples were collected at up to six different time periods equally spaced over a one-year period, with the range of urinary OP metabolite values being similar to the levels reported by Hill *et al.* (1995).

Only a handful of studies have been published in the literature that were specifically focused on biomonitoring of children for OP pesticides and their metabolites. These researchers noted that young children may be more highly exposed and are more susceptible to health risks from exposures to OP pesticides than adults because they are undergoing rapid physiological and behavioral development. Furthermore, in comparison to adults, young children: have a larger surface area to volume ratio; have a relatively large brain size as compared to total body mass; take in more air, food, and water on a per unit body weight; and, absorb, distribute, metabolize, and eliminate pesticides differently than adults (Guzelian *et al.,* 1992). Children also engage in specific activities in which they may more likely come into direct contact with contaminated surfaces and objects (Hubal *et al.,* 2000). These child-type activities include: sitting, playing on the floor; eating while roaming around the house; putting hands, objects, toys into the mouth; licking the furniture, pet, floor; etc.

The Minnesota Children's Pesticide Exposure Study (MNCPES) was the first published study to report multipathway pesticide exposures in a population-based sample of children (Quackenboss *et al.,* 2000; Lioy *et al.,* 2000; Adgate *et al.,* 2001). Personal (hand rinse, duplicate diet, time activity diaries and questionnaires, videotape segment), biological (urine), and environmental (indoor/outdoor air, residential surfaces, soil, drinking water) samples were collected to assess children's aggregate pesticide exposure and attempt to identify the critical pathways of exposure. Three first-morning void urine samples were collected on three separate days during the study. The urine samples were then analyzed for metabolites of commonly used OP pesticides (Adgate *et al.*, 2001). Analysis of these urine samples for OP pesticides and their metabolites have shown that children do have a body burden level of OP metabolites (Quackenboss *et al.*, 2000; Lioy *et al.*, 2000; Adgate *et al.*, 2001). While the MNCPES study didn't assess adult exposures, Adgate *et al.* (2001) compared these urinary biomarker levels of OPs for children with the reference levels reported by Hill *et al.* (1995) and found similar ranges for both children and adults.

Fenske *et al.* (2000) collected and analyzed single void urine samples for OP metabolites from 109 children (up to six years-of-age) who lived in an agricultural community in the state of Washington. The children's urine samples were collected at the convenience of the child and parent. From the children's OP pesticide doses derived from this biologic monitoring study, the authors suggested that residents of agricultural communities may be more exposed to pesticides than the general population.

Two studies have compared urinary metabolite levels for all members of a household (Heudorf and Angerer 2001; Krieger *et al.*, 2001). Heudorf and Angerer (2001) examined urinary metabolite concentrations for children and adults living in dwellings that had not been recently treated with OPs (most recent treatment was four years prior). These investigators suggested that urinary OP metabolite concentrations in children and adults were not different. Krieger *et al.* (2001) assessed the extent of exposures for family members (adults and children) residing

in homes where pesticides have been used. Chlorpyrifos was applied in this study by three different methods: fogger, broadcast, and crack and crevice. Analysis of the family urine samples for OP metabolites showed no significant difference between children and adult exposures for those family members living in the same households. However, both studies only examined the sample results without considering the factors associated with the physiological and behavioral differences between adults and children, a step needed to better describe and understand the real potential for exposure.

Interpreting the results of these published studies presents several challenges. First, only a few studies have been conducted and the results published in the literature. Secondly, the methodologies employed in each study have varied. Only spot urine samples have been collected, and, more importantly, the sample collection times for these spot urine samples have differed for many of the studies, ranging from first-morning voids to convenience samples collected during the morning hours. In the few studies that have collected both the environmental and biological samples, the levels of the OP pesticides and urinary metabolites have not correlated with any of the OP concentrations in the other environmental samples analyzed (Lioy *et al.,* 2000). Some investigators have tried to compare the children's and adult's OP pesticide and metabolite levels without correcting these data for the differences found in children associated with their differences in metabolic rates, muscle mass, creatinine production, and urinary output.

Although the available biological monitoring studies generally indicate children do have a body burden level of OP pesticides, based on the limited number of published studies and the inconsistencies noted above, it is difficult to make any general statements concerning the study population, much less the general population. Equally important, from these limited sets of published data, it is difficult to assess whether children's exposures to OP pesticides are the same, higher, or lower than corresponding adult exposures.

Several relatively large-scale children's aggregate pesticide exposure studies which include OP pesticides are ongoing or near completion by the U.S. EPA National Exposure Research Laboratory (NERL) scientists and academicians. However, the analyzed and published results of this research will not be available for several years. Without these additional data, questions regarding whether children's exposures to pesticides are higher than adults or the validity of the cumulative exposure estimates can not be readily answered.

E. Exposure Characterization Summary

The cumulative exposure assessment of OP pesticides represents the first probabilistic assessment of multichemical and multipathway exposures to pesticides. Estimates of residues in food are based on actual monitoring and consumption data that capture the major food groups consumed by children. Several age groups are defined such that they reflect an adequate number of individuals in each age group and are based on real differences in age-related eating patterns. Estimates from food dietary intake are considered to confidently approximate dietary food exposure of children to OP pesticides. There is also confidence that the dietary drinking water exposure assessment for OP pesticides does not understate potential exposure to children (or any age group) given that regional water exposure assessments were conducted that were designed to focus on areas where combined OP exposure is likely to be among the highest within the region as a result of total OP usage and vulnerability of the drinking water sources. Furthermore, the cross check of PRZM and EXAMS predicted estimates with actual drinking water monitoring data gives confidence in the drinking exposure assessment. Finally, the residential and nonoccupational exposure estimates are also considered to provide protective estimates of children's exposures given the quality of the data and the conservative assumptions used. In summary, there is a high degree of confidence in the exposure data and methodologies used when assessing cumulative risk to children, that are considered to be protective of children without understating risk. Thus, for the exposure assessment, reliable data show that it is not necessary to retain the default 10X special FQPA safety factor.

IV. INTEGRATIVE ANALYSIS OF HAZARD AND EXPOSURE

A weight-of-evidence analysis has been conducted to determine the completeness of the toxicity and exposure databases, and the degree of concern for pre- and postnatal toxicity associated with the common mechanism of toxicity, AChE inhibition. It was determined in the hazard assessment that there are reliable data to support application of a 3X database uncertainty factor (which is used to address the FQPA safety factor provision's expressed concern as to the "completeness of the data with respect to... toxicity to infants and children....") to address the limited data on ChE inhibition in immature animals and evidence that supports the potential for OP pesticides to show ChE inhibitory effects at lower doses in young animal compared to adults (*i.e.*, the age group on which the relative potencies values are based). There is no need for an additional special FQPA safety factor to address potential pre- and postnatal toxicity associated with ChE inhibition because application of the database uncertainty factor to the RPFs for the OP accounts for age-dependent sensitivity in the young and potential neurodevelopmental effects associated with ChE inhibition.

The revised cumulative risk assessment for OP pesticides is based upon the most comprehensive and data-specific exposure assessment ever performed by OPP. This statement is true for all aspects of the exposure estimates including pesticide sources from food, drinking water and residential uses. Each aspect of the assessment relied upon the use of the best available data for input parameters. The data were introduced into the assessment in large part in the form of distributions, permitting the assessment to reflect the full range of variability in each input parameter. This approach deviates from the past practice used particularly for drinking water and residential exposure estimates that relied upon high endpoint estimates. In this assessment, drinking water and residential estimates have been refined in much the same manner previously established for food assessments. The comprehensiveness and thoroughness of this exposure assessment allows OPP to conclude that an additional safety factor is not needed to address the completeness of the exposure database.

While the available data and methodologies used by OPP to estimate exposures cannot be used to precisely define an exact exposure for any given percentile of the population, OPP can bracket or otherwise define the range within which exposures are expected to fall. Specifically, OPP believes that the traditional single-day analysis in which individual days are assessed in isolation reflects a likely upper-bound of exposures. OPP also believes that the actual upper-bound of exposure is lower than the high-end estimated by the rolling average exposure (discussed in the Risk Characterization Section of the OP cumulative risk assessment). Additionally, the cumulative assessment was conducted in a way that does not intrinsically bias the analysis toward over estimation or under estimation of exposures, but instead reflects exposures anticipated to be experienced by the public. Accordingly, OPP believes that the analysis captures the highly exposed groups (including children) and represents exposures reasonably likely to occur and that the above-mentioned "bracketing" represents realistic expected upper and lower bounds on the estimated exposure. Final determinations regarding which predicted exposures will be considered in making a regulatory decision will depend on sensitivity analyses of predicted high-end exposures.

These determinations could also play a role in a final conclusion about whether OPP remains confident that the analysis adequately captures the upper-bound of estimated exposures and, therefore, whether there is continuing support for the conclusion that an additional FQPA safety factor is not needed to address the completeness of the exposure database.

In summary, given the highly refined nature of estimates for all pathways of exposure, the use of bounding estimates to reflect the potential issues associated with timing and repeated exposures, and the application of the database uncertainty factor of 3X, the presumptive 10X safety factor can be removed.

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