A Review of the Reference Dose (RfD) for Chlorpyrifos

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Abstract

Chlorpyrifos is an inhibitor of cholinesterase (ChE), and inhibition of ChE is believed to be the most sensitive effect in all animal species evaluated and in humans from previous evaluations. Recent literature, and in particular epidemiology studies reporting associations between chlorpyrifos levels and fetal birth weight decreases, suggest the need to reevaluate the basis of the Reference Dose (RfD) for chlorpyrifos, however. In this paper, we evaluated newly available publications regarding chlorpyrifos toxicity, and discuss the choice of critical effect--- whether cholinesterase inhibition or developmental effect, the choice of appropriate species and study, the appropriate point of departure, and choice of uncertainty factors---including a discussion of the FQPA safety factor. We conclude that RBC cholinesterase inhibition is the critical effect, that human studies form the best choice of species---supported by a wealth of experimental animal data, that a NOAEL of 0.1 mg/kg-day is the most appropriate point of departure, and that a 10-fold factor for within human variability is sufficient to characterize the overall uncertainty in this rather large database. The resulting RfD is 0.01 mg/kg-day.

Key terms: Chlorpyrifos; Fetal development; ChE inhibition; Risk assessment; Reference Dose (RfD)

1. Introduction

Chlorpyrifos, an irreversible inhibitor of cholinesterase (ChE) including acetylcholine esterase (AChE), is one of the most widely used organophosphate insecticides in the U.S. Sufficient inhibition of AChE in the central and peripheral nervous systems causes excessive accumulation of acetylcholine which in turn results in neurotoxicity in animals and humans. Inhibition of ChE is believed by many groups to be the most sensitive effect in all animal species evaluated and in humans, regardless of route or duration of exposure (e.g., ATSDR 1997; US EPA 2000a; UK, 2003).

Recent developments in the epidemiology database of chlorpyrifos prompted a revisit of the information on this chemical's overall toxicity and corresponding issues in the judgment of its critical effect. For example, Whyatt *et al*. (2004) reported an association between umbilical cord plasma chlorpyrifos levels and fetal birth weight decreases among minority women living in New York City during pregnancy, and this finding raised a concern on whether impaired fetal development could be the critical effect rather than the inhibition of AChE as had been believed so far. Zhao *et al*. (2005) investigated this association through an analysis of other epidemiology and experimental animal studies and showed that the positive association was not consistent with observations from other epidemiology investigations and was also not supported by data from experimental animal studies. Specifically for the experimental animal work, a direct comparison of neonatal information showed that cholinesterase inhibition was a more sensitive indicator of an effect than reduced body weight, and that neonates were equally, or perhaps less, sensitive to cholinesterase inhibition than their maternal parent.

Although other health organizations and investigators have concluded that cholinesterase inhibition is chlorpyrifos' critical effect, not all of these positions have been informed by the recent epidemiology associations. For example, Table 1 summarizes critical effects that were concluded and used by various international health organizations as the basis for developing chlorpyrifos safe doses. Most of these groups feel that inhibition of AChE either in the brain or RBC should be considered the critical effect for chlorpyrifos toxicity. However, many of these assessments were conducted by these organizations before Whyatt *et al*. (2004) published their new findings on fetal developmental effect. Therefore, it becomes important to consider such new information in updating any chlorpyrifos assessment.

The purpose of this paper is to incorporate the new analysis of critical effect by Zhao *et al*. (2005) and other recent publications on chlorpyrifos dose response assessment to determine a new chronic Reference Dose (RfD), with particular close attention being paid to developmental toxicity from epidemiology and experimental animals studies, and the incorporation of information from human studies in the dose response assessment. In this report, we present a weight of evidence analysis of the available chlorpyrifos toxicology and epidemiology data, and issues associated with chlorpyrifos risk assessment, specifically in dose response assessment.

2. Methods

One of the many risk assessment goals with which a risk assessor is often tasked is to determine what exposure for a chemical might be considered "safe." "Safe" or subthreshold doses have been defined by a number of health agencies worldwide. Many of

the underlying assumptions, judgments of critical effect, and choices of uncertainty factors for "safe" doses are similar among health agencies in estimating these subthreshold doses. Our analysis will follow the US Environmental Protection Agency's (EPA) Reference Dose (RfD) method (Barnes and Dourson 1988; Dourson 1994; US EPA 2002a).

The first step in defining the RfD is to identify the chemical's critical effect(s). US EPA (2004) and Haber *et al*. (2001) define critical effect(s) as the first adverse effect(s), or its known precursor, that occurs as dose rate or exposure level increases. For chlorpyrifos, previous assessments depended on available experimental animal studies and clearly suggested a host of effects related to AChE inhibition in the brain, a primary target organ. However, the newly available epidemiology study (Whyatt *et al*., 2004) suggested a possibly more sensitive developmental effect, decreased birth weight, as the critical effect for chlorpyrifos exposure. Because these data appeared to be conflicting, identifying the critical effect from which to base an RfD is the first, and perhaps, the most important step in any chlorpyrifos risk assessment. Zhao *et al*. (2005) separately analyzed this apparent conflict, and their results are briefly reported here (see below).

The second step in the determination of an RfD is in the choice of appropriate species, study, and point of departure. For this evaluation, we also used US EPA methods, including a review of existing experimental animal and human data, and the use of benchmark dose (BMD) for the comparison of age-related differences in sensitivity to chlorpyrifos in neonates and adult rats. EPA Benchmark Dose modeling software (BMDS) version 1.3 was used to estimate these BMDs, and in modeling, a 20% inhibition of ChE activity from the control mean was used as the benchmark response (BMR). The 20% inhibition was used because it is a generally agreed limit above which the ChE inhibition is

considered abnormal. Other choices are also possible. Among four continuous models available in the BMDS software (Linear, Polynomial, Power and Hill), the Hill model provided the best data fitting. Therefore, all of the results presented in our analysis were estimated by using the Hill model.

The last step in the determination of an RfD is the judgment of the appropriate uncertainty factor based on a review of the information supporting the choice of critical effect, and issues associated with extrapolation from experimental animals to humans or from healthy humans to sensitive humans. For this evaluation, we also used US EPA methods, briefly describing EPA's five potential areas of uncertainty for this judgment, and also its Food Quality Protection Act (FQPA) safety factor (Fenner-Crisp 2001; US EPA 2002b).

3. Results and Discussion

3.1 Step 1: identification of critical effect

3.1.1. Cholinesterase Inhibition

The mechanism of toxicity caused by organophosphate including chlorpyrifos has been well documented (Klaassen 2001). Chlorpyrifos-oxon, the active metabolite of chlorpyrifos, affects the nervous system by irreversibly inhibiting the activity of cholinesterase, the enzyme responsible for the destruction and termination of the biological activity of the neurotransmitter, acetylcholine (ACh). AChE is necessary for the proper functioning of the nervous system. The result of ChE inhibition is the accumulation of free, unbound ACh at the nerve endings of all cholinergic nerves. Accumulation of ACh leads to cholinergic over stimulation. The signs of toxicity include those resulting from

effects on the CNS as well as those resulting from stimulation of muscarinic receptors of the autonomic nervous system, and the junctions between nerves and muscles. Without a proper treatment, the toxicity will persist until sufficient quantities of new cholinesterase enzyme are synthesized to destroy the excess neurotransmitter. In this chain of events, inhibition of ChE is the first event, and it is the immediate precursor for the accumulation of ACh.

Besides the effects on nervous system due to inhibition of cholinesterase, chlorpyrifos can also cause other toxicity, but at higher dose levels (U.S. EPA 2000a). Other systemic toxicity caused by chlorpyrifos included body weight loss, decreased food consumption, liver, kidney and adrenal pathology. Neither the rat nor the mouse carcinogenicity studies showed evidence of carcinogenicity, and the mutagenicity studies showed positive response only in an in vitro cytogenic assay in the presence of metabolic activation. Developmental toxicity studies showed that chlorpyrifos caused developmental effects, such as minor skeletal variations, delayed ossification and reduced fetal weight and length in mice and rabbits, and decreased body weight gain and food consumption, reduced pup viability, delays in development, decreased brain weight and morphometric alterations in the brain in rats. However, all these developmental effects were observed in the presence of maternal toxicity. Similarly, chlorpyrifos induced reproductive toxicity in one generation of rats including reduced pup weights and increased pup mortality at dose levels that induced parental toxicity.

In recent assessments (ATSDR 1997; US EPA 2000a; van Gemert *et al*., 2001; UK ACP 2003), chlorpyrifos has been evaluated for toxicity after oral administration in humans, rats, mice, and dogs. A brief excerpt of these results is summarized in Figures 1

and 2. In all experimental animal species, the most sensitive indicator of effect is inhibition of plasma, red blood cell (RBC), and brain ChE, and significant inhibition of plasma and RBC ChE occurs at doses below those that cause brain ChE inhibition. For example, ChE inhibition in the plasma and RBC exceeded 20% in both male and female rats after a 2-year dietary exposure to chlorpyrifos at 1 mg/kg-day and above, but not at 0.1 mg/kg-day (McCollister *et al*., 1974). In dogs treated with chlorpyrifos for 1 or 2 years, plasma ChE activity was significantly and dose-dependently decreased in both sexes at ≥0.03 mg/kg-day, but not at 0.01 mg/kg-day, with significant RBC ChE inhibition occurring only at 0.1 mg/kg-day for some time intervals (McCollister *et al*., 1974). Thus, dogs appear to be the most sensitive experimental animal species for ChE inhibition and systemic effects following chronic exposure.

In addition to the chronic animal studies, a repeated exposure study was conducted in human subjects by Coulston *et al*. (1972) on chlorpyrifos toxicity. In this study, sixteen healthy male adult volunteers were divided into 4 groups of 4 and given oral doses of chlorpyrifos in tablets at 0, 0.014, 0.03 or 0.1mg/kg/day, for 49, 28, 21, 9 days, respectively. RBC ChE (exclusively as AChE in humans) was not inhibited at any dose level. As shown in Figure 3, plasma ChE activity was not consistently affected at 0.014 mg/kg/day. At 0.03 mg/kg/day, this activity was slightly reduced on days 1 and 3, and more reduced on days 16, 18 and 20 although no value reached statistical significance. However, at 0.1 mg/kg/day, the activity was statistical significantly reduced up to 64%. Plasma ChE activity returned to baseline values after 3 weeks at 0.03 mg/kg/day and within 4 weeks at 0.1 mg/kg/day. One of the subjects in 0.1 mg/kg group was removed from the study due to “cold” symptoms (runny nose, blurred vision, and a feeling of

faintness); however, the study authors (Coulston *et al*., 1972) and an expert panel ((Clegg and van Gemert, 1999) judged these signs and symptoms to be unlikely to have been induced by cholinesterase inhibition.

Similar, but less severe, ChE response as observed in the repeated-dose study was also seen in humans after single dose treatment. In the study conducted by Nolan *et al*. (1984) in which six males were orally administered chlorpyrifos at a dose of 0.5 mg/kg, no clinical signs of toxicity or RBC ChE inhibition were observed following treatment. However, plasma ChE activity at 12 and 24 hours post dosing was inhibited by about 70% following the oral dose, but the activity recovered at about 4% per day between days 2 and 14 and 2% per day between days 16 and 30. Kisicki *et al*. (1999) also studied male and female volunteers (six/sex/dose) given a single oral dose of 0.5, 1.0 or 2.0 mg chlorpyrifos/kg of body weight. No clinical signs of cholinergic toxicity were exhibited by the volunteers. No statistical differences or treatment-related changes in RBC ChE activity were identified in any volunteer given the 0.5 or 1.0 mg/kg dose level or in 11 of 12 volunteers given the 2.0 mg/kg dose. However, one volunteer given the 2.0 mg/kg dose had greater than 17.3% decrease in RBC ChE activity in blood specimens collected 8, 12, 24, 36 and 48 hours post-treatment. The study authors concluded that the no-observed-adverse-effect-level (NOAEL) for RBC ChE inhibition for a single dose in fasted humans was 1.0 mg/kg based on the inhibition of RBC ChE activity observed in the single volunteer given 2.0 mg/kg chlorpyrifos/kg body weight dose.

Based on the animal and human studies, as summarized in Figures 1 and 2, dogs appear to be more sensitive to the toxicity of chlorpyrifos than humans based on ChE inhibition in the plasma or RBC, the most sensitive endpoints. From these figures, humans

appear at least as sensitive as rodents to RBC ChE inhibition, but are more sensitive to plasma ChE inhibition than rodents by no more than 3-fold.

3.1.2 Developmental Effects

In a recent epidemiology study, Whyatt *et al*. (2004) reported an association between umbilical cord plasma chlorpyrifos levels and fetal birth weight decreases among minority women living in New York City during pregnancy. This finding raised a concern on whether impaired fetal development could be the critical effect rather than the inhibition of AChE as has been previously judged (as described above), and whether developing fetus or neonates are more sensitive to chlorpyrifos than adults. To examine the relative sensitivity of the developing fetuses and neonates in response to chlorpyrifos, Zhao *et al*. (2005) analyzed fetal body weight changes reported by Whyatt *et al*. (2004) and cholinesterase inhibition from different experimental animal species. This analysis is shown briefly below.

Fetal Body Weight Changes

Chlorpyrifos not only can cause neurotoxic effect through inhibition of cholinesterase activity, but also can cause other systemic toxicities, such as maternal toxicity in the treated pregnant dams and their fetuses, although the developing fetuses are not considered as more sensitive to chlorpyrifos than the adult animal (ATSDR, 1997; EPA, 2000a; van Gemert *et al*., 2001; UK ACP, 2003). In the Whyatt *et al*. (2004) study, an association was reported between umbilical cord plasma chlorpyrifos levels and fetal birth weight decreases among minority women living in New York City during pregnancy. The authors stated that their results "*indicate that prenatal chlorpyrifos exposures have*

impaired fetal growth among this minority cohort and that diazinon exposures may have contributed to the effects." This finding raised a legitimate concern as to whether impaired fetal development could be the critical effect rather than the inhibition of AChE because the exposure level in the studied subjects was much lower than that expected to cause ChE inhibition. This finding of an association does not establish causality, of course, and the authors acknowledge that additional research is needed to either support or refute their findings.

Fortunately for chlorpyrifos, additional research is available including epidemiologic evidence and relevant toxicological data from animal studies, as summarized by Zhao *et al*. (2005). These investigators found that the positive association observed in Whyatt *et al*. (2004) study was inconsistent with the results of two other epidemiology investigations (Berkowitz *et al*., 2004 and Eskenazi *et al*., 2004). Moreover, a direct comparison of experimental animal neonatal information showed that cholinesterase inhibition is a more sensitive indicator of effect than reduced body weight, and that neonates repetitively exposed to chlorpyrifos are equally, or perhaps less sensitive to cholinesterase inhibition than their maternal parent. Based on a review of the available human studies and comparison of human cord blood chlorpyrifos concentrations with blood chlorpyrifos concentrations that in animals caused effects with good dose-response, it appears unlikely that the exposure level encountered by the population reported in Whyatt *et al* (2004) study would cause significant fetal developmental effect. Thus, weight of evidence on fetal developmental toxicity from experimental animals and humans suggests that the critical effect for chlorpyrifos still appears to be cholinesterase inhibition (Zhao *et al*., 2005).

Cholinesterase Inhibition:

Another way to evaluate the relative sensitivity of developing fetuses and neonates is to examine the most sensitive response in various age groups, *i.e.*, cholinesterase inhibition as has been identified so far. In a developmental study (Mattsson *et al*., 2000), pregnant rats were exposed to chlorpyrifos from GD 6 through postnatal day (PND) 10. As discussed by Zhao *et al*. (2005), in dams, ChE activity was significantly inhibited at ≥0.3 mg/kg/day in the plasma and RBCs and at ≥1 mg/kg/day in the brain (fore- and hindbrain), whereas in pups, ChE inhibition was only observed in these tissues at 5 mg/kg/day. Not only was the extent of ChE inhibition in dams greater than that in fetuses/pups, but the inhibition in dams also occurs at doses >3x lower than in fetuses. Thus, as summarized in Table 2, dams are more sensitive than fetuses to chlorpyrifos exposure during pregnancy.[1]

Zheng *et al*. (2000) also investigated the age-related differences in sensitivity to chlorpyrifos in neonates (immature) and adult rats after direct oral dosing. Neonatal (7-day old) and adult (90-day old) rats were administered chlorpyrifos via gavage at doses of 0, 0.15, 0.45, 0.75, 1.5, 7.5, or 15 mg/kg/day for 14 days and sacrificed either 4 hours after the first dose or the 14th dose. After repeated dosing, signs of chlorpyrifos toxicity were observed as evidenced by significant mortality at 15 mg/kg/day in neonates; autonomic dysfunction at ≥7.5 mg/kg/day in neonates vs. 15 mg/kg/day in adults; and statistically significant decrease in pup and adult body weight at ≥7.5 mg/kg/day and ≥15 mg/kg/day, respectively. Plasma, RBC, and brain ChE activities were significantly

[1] Comparisons of all data from the Mattsson *et al*. (1998) study are available at www.tera.org.

inhibited at 1.5 mg/kg/day in neonates and at 0.75, 0.45, and 7.5 mg/kg/day in adults, respectively (summarized in Table 2), indicating that neonates are less sensitive to repeated chlorpyrifos exposures than are adults, as measured by the critical effect, plasma and RBC ChE inhibition.

In contrast to repeated dosing results, following acute chlorpyrifos exposure (the first dose), more extensive ChE inhibition was noted in neonates than in adults (especially in the brain), with NOAELs based on ChE inhibition in adult tissues being 1-(doubled dose) to ≥10-fold higher than those in neonates (Zheng *et al*., 2000). In the same study, neonates and adult rats were exposed acutely to chlorpyrifos at doses of 3-100 mg/kg and 120-400 mg/kg, respectively, and results based on lethality indicated that neonates are about 9-fold more sensitive to chlorpyrifos exposure than are adults. Similar results that indicate neonates appear to be more sensitive/susceptible to acute lethality from chlorpyrifos than adult rats have been reported (Pope *et al*., 1991), while juvenile rats are also intermediate in sensitivity (Moser and Padilla, 1998).

To facilitate the comparison of Zheng *et al*. (2000) data, we estimated benchmark doses (BMDs) for each age group, and then directly compared these BMDs to analyze the relative sensitivity of each age group. Because the BMD is based on the complete dose response assessment curve, it is less influenced by individual data variability; thus, a BMD comparison would represent a truer difference among dose-response relationships rather than a direct comparison of NOAELs. The BMD comparison between neonates and adults are summarized in Table 3.

Similar to the finding from a direct comparison of NOAEL levels for various endpoints, when the benchmark dose response (BMR) was defined as 20% ChE inhibition

(Table 3), BMDs were similar in neonates and adults repeatedly exposed to chlorpyrifos, for all three tissues (the plasma, RBC, and brain) evaluated. In contrast, following acute exposures, neonates had an RBC BMD comparable to adults, but the BMDs in the brain and plasma were 4 times lower in neonates than in adults; *i.e*., neonates appear to be more sensitive. Therefore, similar to the finding by Zheng *et al*. (2000), our BMD analysis indicates that neonates are equally or less sensitive to repeated chlorpyrifos exposures than are adults, as shown by all three measures of ChE inhibition, and that neonates are more sensitive to acute exposure to chlorpyrifos than are adults for at least two of three measures of cholinesterase inhibition. However, we find a smaller difference in sensitivity in terms of BMD values between these two age groups when compared with the results of Zheng *et al*. (2000).

Available data on age-related ChE sensitivity to chlorpyrifos suggest that the difference in sensitivity might be due to the rate of recovery of ChE. As indicated by Mortensen *et al*. (1998), brain AChE activity showed an age-related increase in Vmax until postnatal day 17 with no change in Km in terms of response to chlorpyrifos treatment. The 50% inhibition concentrations (IC50) for postnatal day 4 and adult brain AChE were virtually identical. Thus, sensitivities of AChE in young and adult brain to low dose chlorpyrifos are not different. However, there is more rapid recovery of ChE activity in neonatal compared to adult tissues (Liu *et al*., 1999; Moser and Padilla, 1998; and Pope *et al*., 1991). Thus, the greater synthesis of new ChE molecules following each exposure may allow the neonate to tolerate relatively higher repeated exposure to chlorpyrifos.

Table 3 indicates that relative to acute exposure, repeated dosing provides BMD estimates that are either generally comparable (*i.e*., neonatal plasma ChE) or lower (*i.e*.,

RBC or brain ChE) values. Thus, based on available information as summarized in Table 2 & 3, the most sensitive responses occurred after repeated dosing rather than single dosing, and based on comparison of these most sensitive responses, i.e., ChE inhibition in the plasma or RBC, neonates are not more sensitive than treated adults. According to the assumption used by U.S. EPA's noncancer risk assessment that preventing the critical effect will also prevents against all the effects, using the ChE inhibition in the plasma or RBC after repeated exposure as the point of departure will provide a more conservative estimate of the risk value.

Sensitivity of Brain Developmental Effects

In addition to the effects on ChE inhibition and fetal body weight changes, chlorpyrifos also affects neonatal brain development at high doses, but not at lower doses (Campbell *et al*., 1997). For example, at 1 mg/kg/day, chlorpyrifos affects neural cell development in young rats when administered subcutaneously during pregnancy or to neonates (Dam *et al*. 1998; Johnson *et al*. 1998; Song *et al*. 1998). Recent studies involving chlorpyrifos exposure during early and late gestation have also shown that chlorpyrifos elicits both short- and long-term changes in serotonin (5HT) systems, disrupting the ability of 5HT to modulate adenylyl cyclase (Aldridge *et al*. 2003; Qiao *et al*., 2004). In the brainstem, these changes are observed at doses (≥10 mg/kg/day) exceeding the threshold for systemic toxicity such as decreased body weight (Qiao *et al*. 2002), whereas a much larger effect was evoked in the forebrain at ≥1 mg/kg/day. Gestational exposure via oral route at doses up to 7 mg/kg/day only produced short-term inhibition of pup brain AChE activity and muscarinic cholinergic receptor levels.

However, exposure to 7 mg/kg/day caused long-term alterations in presynaptic cholinergic neurochemistry, such as choline acetyltransferase (ChAT) activity, high-affinity choline uptake –HACU- transporter levels, and synaptosmal levels of the vesicular acetylcholine transporter (VAChT) (Richardson and Chambers 2003, 2004).

It is important to note that in many of the parenteral administration studies, dimethyl sulfoxide (DMSO) was used to ensure rapid and complete absorption (Whitney *et al*. 1995). Consequently, blood chlorpyrifos concentrations may likely be higher than those reached following oral administration. For example, 1 mg/kg dose of chlorpyrifos administered subcutaneously in DMSO will be rapidly and completely absorbed and evenly distributed throughout the body, temporarily causing potential levels as high as 1000 ng chlorpyrifos /g of body tissue. However, the human volunteer study by Nolan *et al*. (1984) reported that oral administration of 0.5 mg/kg or dermal administration of 5 mg/kg yielded blood chlorpyrifos concentrations of ≤30 ng/g and ≤10 ng/g, respectively. This suggests that subcutaneous administration with DMSO may result in effective blood chlorpyrifos levels that are several folds higher than the dose levels used, for example, in oral chlorpyrifos toxicity studies. In addition to the absorption issue, DMSO has also been shown to have anticholinesterase activity (Brayton, 1986) and to affect central (Fossom *et al*. 1985) and peripheral nervous systems (Calvetti *et al*. 2000). Therefore, using DMSO as a solvent might also confound the observations made by some authors (*e.g*., Campbell *et al*., 1997; Dam *et al*., 1998; Johnson *et al*., 1998; Song *et al*., 1998; Qiao *et al*., 2002). Thus, the potential chlorpyrifos-induced brain developmental effects are likely to occur at the oral doses higher than those causing ChE inhibition in the blood plasma and RBCs.

Thus, as discussed above, weight of evidence on fetal developmental toxicity from animals and humans suggests that the critical effect for chlorpyrifos still appears to be cholinesterase inhibition. Neonates are equally or less sensitive to repeated chlorpyrifos exposures, as measured by ChE inhibition, than are adults. The data on brain developmental effects after parenteral administration of chlorpyrifos also suggested that the potential chlorpyrifos-induced brain developmental effects are likely to occur at oral doses higher than those causing ChE inhibition in the blood plasma and RBCs. Therefore, the wealth of information all indicates that the critical effect is ChE inhibition.

3.2. Step 2: What is the Choice of Appropriate Species and Study?

EPA's policy when developing RfDs in many of its programs, regional offices, and Office of Research and Development (ORD) has been to use human data first and foremost in the determination of critical effect and choice of uncertainty factors. The preference for use of human data is found in many EPA publications, risk positions, risk methods documents, and practice (*e.g.*, Barnes and Dourson, 1988; EPA, 2002a). The available human studies on chlorpyrifos toxicity provided information regarding relative sensitivity of humans to the pesticide compared to experimental animals without causing serious or irreversible harm to the subjects, and such information could not be reasonably obtained by other means. As concluded by Resnik and Portier (2005), such studies should be permitted in risk assessment.

Several human studies exist that monitored for the inhibition of ChE, the critical effect, and while each may have some difficulty, the synthesis of their individual results leads to an adequate and consistent picture of chlorpyrifos toxicity. This picture does not

include results in potentially more sensitive humans. However, a wealth of experimental animal research is available that supports and enhances the human data, and that also includes potentially more sensitive subgroups. The available database including animal and human studies supports using human data as the point of departure in deriving the chlorpyrifos "safe" dose.

The available data indicate that the chlorpyrifos-induced effects in humans are comparable to that in experimental animals. For example, Figure 1 shows the NOAELs of chlorpyrifos-induced RBC ChE inhibition across different species including human, rat, mouse, and dog. The NOAEL in humans (0.1 mg/kg/day) was comparable to that in the rat and mouse (0.1 and 0.13 mg/kg/day, respectively), but was higher than that in dogs [0.01 (Barker, 1989) and 0.03 mg/kg/day (reported as significant inhibition in female rats in the original report by McCollister *et al*., 1974)]. U.S. EPA considered NOAEL for RBC ChE inhibition to be 0.03 mg/kg/day. However, a more recent publication by Mattsson *et al*. (2001) showed that a statistical reanalysis of the original data (McCollister *et al*., 1974) indicated a NOAEL of 0.1 mg/kg/day. The low NOAEL in the 90-day dog study (Barker 1989) was most likely due to the large dose span between the NOAEL of 0.01 mg/kg/day and the LOAEL of 0.22 mg/kg/day. However, the chronic dog study with smaller dose span showed that the NOAEL for RBC ChE inhibition was at 0.03 mg/kg/day based on the original report, which was lower than that in humans, or 0.1 mg/kg/day based on more recent reanalysis, suggesting that the dog is more sensitive than, or at least comparable to, human. In addition, the human NOAEL was free standing with no RBC ChE inhibition observed at the highest dose tested. Thus, the actual subthreshold dose might be even

higher. Therefore, based on RBC ChE inhibition data, the human is at least comparable, if not less sensitive, than some experimental animals.

Three human studies have been conducted collectively using both male and female volunteers (Coulston *et al*., 1972; Nolan *et al*., 1984; Kisicki *et al*., 1999). There were some limitations in these studies. For example, all these human studies were limited by relatively small sample size, and in some studies (Coulston *et al*., 1972; Kiskcki *et al*., 1999) only single sex of subjects was tested. Please note that the small sample size was also an issue with dog chronic studies (McCollister *et al*., 1974). In addition, Coulston *et al*. (1972) study was conducted before implementation of good laboratory practice (GLP), while Kisciki *et al*. (1999) study was conducted in accordance with GLP as well as all applicable U.S. clinical study guidelines. Unfortunately, the results from these latter two studies were not published in peer review journals, but they have been made available to the public through U.S. EPA. In spite of these limitations, the results from all these studies are mutually supportive and consistent. They tested otherwise average healthy individuals of both sexes through an appropriate dose range of interest. Data from these studies suggest a NOAEL for RBC ChE inhibition after repeated exposure of 0.1 mg chlorpyrifos/kg (see below).

3.3. Step 3: Point of Departure Analysis

The NOELs from the studies in different species are summarized in Figures 1 and 2. The available data indicated that in terms of the response to chlorpyrifos-induced plasma ChE inhibition, human is comparable to dog, but is more sensitive than the rat and mouse.

It is worth noting that RBC contains only AChE while plasma contains both butyryl cholinesterase (BuChE) and AChE in varying ratios depending upon the species. While human plasma ChE is overwhelmingly BuChE, the dog plasma has majority of BuChE with a ratio of BuChE to AChE of 7:1 (Scarsella *et al*., 1979) and rat plasma contains approximately 50% or more of AChE with a BuChE to AChE ratio of 1:3 in males and 2:1 in females (Edwards and Brimijoin, 1983). BuChE significantly differs from AChE in substrate affinity, chemical structure, and sensitivity to chlorpyrifos inhibition, and the rate constants for mammalian BuChE inhibition by chlorpyrifos-oxon are 160- to 750-fold larger than those of AChE from the same species (Amitai *et al*., 1998). This marked difference in inhibition kinetics between these two forms of ChEs may contribute to the observed differences in the levels of inhibition between plasma ChE and RBC ChE in humans and experimental animals due to different composition of ChE in these tissue compartments. The difference in the composition of plasma ChE in different species also further indicates the importance of using human data in deriving a safe chlorpyrifos dose. Since AChE significantly differs from BuChE, AChE (the form of ChE in the RBC) inhibition, but not BuChE (the primary form of ChE in the plasma, especially in humans) inhibition, is a more appropriate basis for deriving the chlorpyrifos "safe" dose.

Inhibition of blood ChE (*i.e*., plasma and RBC) is not itself an adverse effect according to some authorities (US EPA, 2000b), but may indicate a potential for adverse effects on the nervous system. As indicated by Norstrandt *et al*. (1997), at a chlorpyrifos dose level (10 mg/kg) that caused no behavioral effects, AChE activity was reduced by 92% in the RBC, 41% in the brain, 39% in the retina and 56% in the heart. At a higher dose level that caused behavioral effects (30mg/kg), AChE inhibition was 96% in the

444 RBC, 71% in the brain, 65% in the retina and 67% in the heart. Thus, the measurement of
445 AChE inhibition in the RBC is more sensitive than that in target tissues, *i.e.*, the brain,
446 heart, or voluntary muscle. At any given dose level, RBC ChE is always inhibited to a
447 greater extent than the AChE in the target tissues. In the absence of the information on
448 inhibition of the target tissue AChE in humans and experimental animals, RBC ChE data
449 could be considered appropriate surrogate or precursor, although it is a more sensitive
450 estimate (van Gemert *et al*., 2001).

451 To support the comparison between the 20-day human study and chronic animal
452 studies, we summarized the time-course of the chlorpyrifos-induced ChE inhibition in dog
453 plasma (see Figures 4 and 5). At the lowest-observed-effect level (LOEL) of 0.1
454 mg/kg/day, there was no significant change, indicating a variation of less than 20%, in the
455 intensity of ChE inhibition during 1 year of chlorpyrifos treatment. Even at the two higher
456 doses (1 and 3 mg/kg/day), there was no trend of increase in ChE inhibition during
457 chlorpyrifos treatment. Thus, no increased response was expected in longer than 20 days
458 of treatment. We expect the same may be true with the human response, *i.e.*, a 20-day
459 human study is comparable to the potential longer-term human exposure, although this
460 supposition has some uncertainty.

461 The Coulston *et al*. (1972) study might be used as the critical study for deriving a
462 chronic RfD. Although this study had small sample sizes (n=4), it was comparable to the
463 sample size of 3-4 dogs/sex/dose used in the dog study (McCollister *et al*. 1974). In
464 addition, a lack of inhibition of RBC ChE activity is seen at higher doses of chlorpyrifos,
465 0.5 mg/kg and 1.0 mg/kg, in the acute human studies (Nolan, *et al*. 1984; Kisicki *et al*.
466 1999). These results further support the observation of the NOAEL of 0.1 mg/kg/day in

the humans during repeated exposure (Coulston *et al*. 1972). Considering the usual choice of a good quality human study over an animal study in deriving RfDs (Barnes and Dourson, 1988), the Coulston *et al*. (1972) human study should be preferred to McCollister's dog study in deriving the chronic chlorpyrifos RfD. The dog data should in turn be considered as highly supportive.

3.4. Step 4: Areas of Uncertainty in Safe Dose Assessment

In the process of non-cancer risk assessment by US EPA (2002a), five different uncertainty factors were suggested to address issues of variability and uncertainty. Among them, interspecies and intraspecies uncertainty factors are used to address the uncertainty between experimental animals and humans, and the variability within different human populations. Three other factors (Subchronic, LOAEL, Database) are used to address lack of information. Typically, the maximum composite uncertainty factor that US EPA will apply is 3000.

3.4.1. Interspecies Variability (UF_A):

This factor accounts for the differences that occur between animals and humans when animal data are used as the point of departure. It is considered to be composed of subfactors for toxicokinetics (how the body distributes and metabolizes the chemical) and toxicodynamics (how the body responds to the chemical). If no information is available on the quantitative differences between animals and humans in either these two subcomponents, then a default value of 10 is used. If information is available on any of these two subcomponents, then this information is used along with a default value of 3 for

the remaining subfactor. If an RfD is based on human data, then a value of 1 is appropriate for this factor.

When the human data from Coulston *et al*. (1972) study is used as the point of departure to derive the risk value, a value of 1 is appropriate because no extrapolation from animals to humans is necessary. However, one may wish to use the chronic dog study (McCollister *et al*. 1974) as the critical study. As summarized above, the similarity in chlorpyrifos-induced ChE inhibition in both the plasma and RBC between humans and animals indicates that humans are no more sensitive to chlorpyrifos than tested animals, at least for dogs. Therefore, when a NOAEL from a dog study is used to derive an RfD, we suggest the use of an uncertainty factor of less than a default value of 10, and based on the data shown in Figure 6, perhaps a value of 1-fold should be used.[2]

3.4.2. Intraspecies Variability (UF_H):

The factor for intraspecies variability accounts for the natural differences that occur between human subpopulations and for the fact that some subpopulations may be more sensitive than the average population. Similar to the interspecies uncertainty factor, this factor is composed of two subfactors for toxicokinetic and toxicodynamic differences. If no information is available on human variability, then a default value of 10 is used. However, if adequate information is available on any of the two subcomponents, then this information is used along with a default value of 3 for the remaining subfactor. If an RfD is based on human data gathered in the known sensitive subpopulation, a value of less than 10, perhaps even 1, may be chosen for this factor.

[2] Figure 6 compares BuChE, while the critical effect is considered to be RBC ChE, because comparative data are available for BuChE but not RBC ChE. This results in some additional uncertainty.

Toxicokinetic variation could be due to variations in absorption, distribution, metabolism and excretion. Available data indicates that paraoxonase (PON1) plays a major role in detoxification of the chlorpyrifos active metabolite, chlorpyrifos-oxon. A human genetic polymorphism in the PON1 gene results in the expression of a range of PON1 enzyme activity within a human population. This variation in the PON1 enzyme activity could contribute to toxicokinetic variation in humans. Based on analysis by Timchalk *et al.* (2002), such contribution to the variation in the chlorpyrifos-oxon metabolism in the brain would be significant at the dose levels higher than 0.5 mg/kg, but not at the level of 0.005 mg/kg which is close to the estimated RfD. According to IPCS (2001) guideline on chemical specific adjustment factor, the relative insensitivity of PON1 enzyme activity to the genetic polymorphism in the PON1 gene suggests that factors other than polymorphism in the PON1 gene might be responsible for the chlorpyrifos toxicokinetic variation in humans. However, there is no quantitative data could be used to assess such variation.

Since the preferred human critical study (Coulston *et al*. 1972) is based on healthy humans, and there is no conclusive information about relative sensitivity of other human subpopulations, such as developing fetuses, to chlorpyrifos exposure, a default value of 10 is recommended. Similarly, a default UF of 10 for this area of uncertainty should also be used if the chronic dog study (McCollister *et al*. 1974) is considered as the critical study.

3.4.3. Subchronic to Chronic Extrapolation (UF_S).

Because the RfD is intended to protect for a lifetime exposure, and chronic exposure might result in more severe adverse effects, this factor is applied when the

database lacks information on the health effects of the chemical following chronic exposure. This factor is chosen based on two considerations: 1. Are there data demonstrating other, more sensitive, health effects following chronic exposure when compared with shorter term exposure? 2. Are there data demonstrating that the critical effect(s) progresses in severity as exposure duration increases or that its NOAEL or other point of departure decreases in value? If the database contains no information on chronic exposures, a default value of 10 is often applied to data from a shorter-term study, unless other data suggest a lack of progression with exposure duration. If the database contains adequate chronic bioassays, then a value of 1 is generally appropriate. If there are data addressing only one of the two issues, then a default of 3 may be applied.

If we base a chlorpyrifos RfD on the shorter-term human studies rather than the chronic dog studies, then we should consider whether an uncertainty factor for subchronic to chronic extrapolation is needed. Figures 4 and 5 show the time-course of the chlorpyrifos-induced plasma ChE inhibition in a one-year dog study (McCollister *et al*. 1974). In either Figure 4 or 5, at the LOEL of 0.03 mg/kg/day, there was no significant change, indicating a variation of less than 20%, in the degree of ChE inhibition as exposure duration increased during 1 year of chlorpyrifos treatment. Even at the three higher doses (0.1, 1, and 3 mg/kg/day), there was no trend of increase in ChE inhibition during chlorpyrifos treatment. Nor were increases in inhibition noticeable as exposure duration increased with the one lower dose of chlorpyrifos. Additionally, no increased response in plasma ChE inhibition was evident as duration increased. This pattern is also apparent when RBC ChE inhibition is plotted in female or male dogs.

A similar pattern for plasma ChE inhibition is also seen in the available human data, with a much more limited exposure duration of 21-28 days (Figure 3). Thus, although the length of the human study is not strictly comparable to the longer-term dog exposure, the critical effect, ChE inhibition, does not exhibit a trend that would suggest lower NOAELs as duration increases, an observation consistent with the dog data. This observation significantly reduces the scientific need for an uncertainty factor to account for the short exposure of the human study. Therefore, if human data are used as the basis for the RfD, an uncertainty factor for subchronic to chronic extrapolation of 1-fold or perhaps 3-fold appears reasonable.

3.4.4. LOAEL to NOAEL Extrapolation (UF_L).

Since the RfD is considered to be a subthreshold value that protects against any adverse health effects, this factor is applied when the critical study lacks information to identify a NOAEL. If the critical study does not identify a NOAEL, then a default of 10 is used for this factor. Otherwise, a value of 1 is appropriate. Often, if the critical study does not identify a NOAEL, but the adverse effects observed are of minimal severity, then a default of 3 will be considered appropriate for use of a "minimal LOAEL". For chlorpyrifos assessment, if a NOAEL of 0.1 mg/kg/day for RBC AChE inhibition in the humans after repeated exposure (Coulston *et al*. 1972) is used as a point of departure, no extra uncertainty factor is needed. Therefore, the appropriate value for this factor is 1. The same factor is also appropriate for a point of departure based on the NOAEL from the dog study.

3.4.5. Database (UF_D).

Based on US EPA's non-cancer risk assessment methodology, the database for deriving a high confidence RfD should include a minimum two chronic bioassays testing systemic toxicity by the appropriate route of exposure in different species, one two-generation reproductive toxicity study, and two developmental toxicity studies in different species. The minimal database required for deriving an RfD is a single subchronic bioassay, which includes a full histopathology examination. The database factor is used when a potentially more sensitive health effect may not be identified if the database is missing a particular type of study. This factor may also be used if the existing data indicate the potential for a heath effect, for example, neurotoxicity or immunotoxicity, but this effect is not fully characterized in the available standard bioassays. If the database is complete for deriving a high confidence RfD, a value of 1 is considered appropriate. Otherwise, a default factor of as high as 10 is used.

The database for chlorpyrifos includes a large number of experimental animal studies, including multiple chronic studies in several species (Figure 1 & 2), numerous shorter-term bioassays, developmental toxicity studies in various species (e.g., Deacon *et al*. 1980; Breslin *et al*.; 1996, Rubin *et al*., 1987a,b), and 1- or 2-generation reproduction studies (e.g., Mattsson *et al*., 2000; James *et al*., 1988; Breslin *et al*., 1991, 1996). The database also includes human clinical, epidemiology, and occupational studies. The weight of evidence from all of these studies suggests that inhibition of ChE is the most sensitive effect in all animal species evaluated and in humans, regardless of route or duration of exposure, and humans are no more sensitive to chlorpyrifos than the most sensitive non-human species tested, the dog. Moreover, a recent evaluation of either birth weight

decrease or cholinesterase inhibition as a critical effect reaffirmed the latter as being critical (Zhao *et al*., 2005). Even though chlorpyrifos can cause neurotoxic effect at high dose, preventing the ChE inhibition would protect humans and animals from further neurotoxic effects. Therefore, the overall chlorpyrifos database appears to be complete, and any new studies that are done to fine tune our knowledge of the chlorpyrifos mode of action will not likely identify lower points-of-departure than can be estimated from the existing database. An appropriate value for this factor is likely to be 1.

In summary, when the human data from repeated exposure to chlorpyrifos are used as the point of departure, the areas of uncertainty for a chlorpyrifos RfD that needs to be addressed by the use of uncertainty factors are human variability, with the possible additional uncertainty due to the length of the available human studies. Our evaluation suggests that for all other areas of uncertainty, including the suggestive results of Whyatt *et al*. (2004) and others, a factor of 1 is appropriate. Thus, a safe dose could be developed from the NOAEL for RBC ChE inhibition in humans (Coulston *et al*. 1972), with a 10-fold, or perhaps 30-fold, uncertainty factor.

3.4.6. Use of FQPA Safety Factor

For the purposes of developing an RfD, a concern exists for the toxicity of chlorpyrifos in neonatal and young animals because of their potentially greater sensitivity than adults. This concern has to be focused on the critical effect, ChE inhibition, and not effects of different severities that occur at higher chlorpyrifos doses. This is because one of the basic assumptions of the RfD is that if the critical effect is prevented, then other more severe effects are prevented as well (Barnes and Dourson, 1988; US EPA 2002a).

Fortunately, a wealth of data and analyses are available on this critical effect in adults, neonatal and young animals. The definitive study for this comparison appears to have been done by Mattsson *et al*. (2000), who specifically tested ChE activity in 5 different organs in dams and their fetuses or pups at 5 different time points, and at 3 different doses and control. A unique aspect of the Mattsson *et al*. (2000) work is that they also measured levels of chlorpyrifos and one of its principal metabolites, TCPy, in the blood of both the dam and corresponding fetus or pup. Thus, direct comparisons of sensitivity (*i.e*., toxicodynamics) to the critical effect between these differently aged groups are possible on a tissue-dose, rather than an administered-dose-specific basis. No other study comparing adult and neonatal chlorpyrifos toxicity has this unique feature.

As analyzed by Zhao *et al*. (2005) and briefly summarized above, the results of the repeated-dose study of Mattsson *et al*. (2000) unequivocally show that neonatal and young animals are equally or perhaps less sensitive than adults to the ChE inhibition on a tissue dose and tissue response specific basis. Similarly, BMD analysis of the Zheng *et al*. (2000) study (Table 3) would suggest that neonatal experimental animals are no more sensitive to repeated exposure to chlorpyrifos than are adults.

In reviewing all of this information, our overall judgment is that an FQPA safety factor is not needed (or at least its toxicity component). This is because:

- The critical effect is considered to be RBC ChE inhibition, and not brain or plasma inhibition. Our BMD analysis of the acute exposures in the Zheng *et al*. (2000) study did not show a difference between the neonatal and adult experimental animals for RBC ChE inhibition.

- Our BMD analysis of the repeated exposures in the Zheng *et al*. (2000) study did not indicate that neonatal experimental animals were more sensitive than adult experimental animals for any ChE inhibition.
- Our toxicodynamic analysis of the Mattsson *et al*. (2000) study unequivocally shows that neonates are not more sensitive than their mothers to ChE inhibition in 5 tissues and for multiple time measurements. See footnote 1 for reference to an analysis of the complete dataset.
- Our review of the overall database for chlorpyrifos indicates that a database uncertainty factor is not needed. EPA (2002b) suggests that an FQPA factor is also not needed when the database factor has been considered.

5.0 Conclusion

Based on the animal and human studies, the most sensitive indicator of effect of chlorpyrifos is inhibition of ChE in target tissues. Of the possible ChE inhibitions in the plasma, RBC, and brain, RBC ChE inhibition is clearly the critical effect. It is both more relevant to human health risk assessment than plasma ChE inhibition because of its closer structural affinity to brain ChE and it precedes the inhibition of brain ChE, an unequivocal adverse effect, as dose is increased. Thus, RBC ChE inhibition fulfills the definition of the critical effect, the first adverse effect or its known precursor (EPA, 2002a). The overall weight of evidence on fetal developmental toxicity from animals and humans suggests that this effect does not precede RBC ChE inhibition, the critical effect for chlorpyrifos. Thus, as long as the RfD is based on the critical effect, developmental toxicity is not expected to occur.

Humans appear less or equally sensitive than dogs, and at least as sensitive as rodents to RBC ChE inhibition and are more sensitive than rodents to plasma ChE inhibition by no more than 3-fold. Moreover, 3 mutually supporting human studies exist on which to base an RfD. Of these 3 studies, a NOAEL of 0.1 mg/kg for RBC ChE inhibition in humans after repeated exposure is considered the most appropriate point of departure to estimate the RfD. The selection of human data from repeated dosing is further supported by longer-term animal studies in multiple species.

After applying an overall uncertainty factor of 10 for intraspecies variability to this NOAEL of 0.1 mg/kg-day, the estimated RfD for chlorpyrifos is 1×10^{-2} mg/kg-day, which is well within the range of values derived by other groups (Table 1) but based on previously available literature. This RfD could be potentially lower if a partial uncertainty factor is used for the subchronic to chronic extrapolation, or potentially higher if the critical effect is considered to be brain cholinesterase inhibition as per the UK ACP (2003). In any case, confidence in this RfD is high because of the mutually supporting information in experimental animals and humans.

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7.0 **References**

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932 Table 1. Summary of Critical Effects Which International Organizations or Investigators

933 Used as the Basis in Derivation of Safe Doses

Organization	**Critical effect**	**Study**	**Uncertainty Factor**	**Safe Dose**
ATSDR, 1997	Plasma and RBC ChE inhibition in rats	McCollister . 1974	10	0.001 mg/kg/day
EPA, 2000a	Plasma ChE inhibition in dogs	McCollister *et al.* 1974	100	0.0003 mg/kg/day
Van Gemert *et al*., 2001	RBC AChE inhibition in humans	Coulston *et al*., 1972; Nolan *et al*. 1984; Kisicki *et al*., 1999	10	0.01 mg/kg/day
Health Canada, 2003	RBC AChE inhibition in 3 species	Yano et al., 2000	100	0.01 mg/kg/d (healthy adults only)
Health Canada, 2003	RBC AChE inhibition in rats	Zheng *et al*., 2000	100	0.00075 mg/kg/day (infants and children)
UK ACP, 2003	Brain AChE inhibition in dogs	McCollister *et al.* 1974	10	0.1 mg/kg/day
WHO, 2004	Brain AChE inhibition in mice, rats and dogs; RBC AChE inhibition in humans	McCollister *et al*., 1974; Coulston *et al*., 1972	100 for animal NOAEL 10 for human NOAEL	0.01 mg/kg/day
Zhao *et al*., 2005 (this study)	RBC AChE inhibition in humans with supporting data in experimental animals	Coulston *et al*., 1972; Nolan *et al*. 1984; Kisicki *et al*., 1999	10	0.01 mg/kg/day

934 Table 2. Effective dose comparison between adult and neonate rats.

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		Brain	RBC	Plasma
Developmental study (Mattsson et al., 2000)	Neonate	5	5	5
	Adult (treated dam)	≥ 1	≥ 0.3	≥ 0.3
	~Adult/Neonate Ratio	0.2	0.06	0.06
Repeated dosing (14-day) study (Zheng et al., 2000)	Neonate (7-day old rats)	1.5	1.5	1.5
	Adult (90-day old rats)	0.75	0.45	7.5
	~Adult/Neonate Ratio	0.5	0.3	5

936 All the doses are in unit of mg/kg/day.

937 Table 3. BMD comparison between neonates and adults when BMR is defined as 20%

938 ChE inhibition [Based on Zheng *et al*. (2000)].

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Exposure Duration	Age Group	Brain	RBC	Plasma
Acute	Neonate	1.5	1.1	0.30
	Adult	5.9	1.4	1.3
	~Adult/Neonate Ratio	4	1	4
Repeated	Neonate	1.2	0.46	0.48
	Adult	1.5	0.33	0.41
	~Adult/Neonate Ratio	1	0.7	0.9

940 All the doses are in unit of mg/kg/day.
941 BMD = benchmark dose (mg/kg/day)
942 BMR = benchmark response
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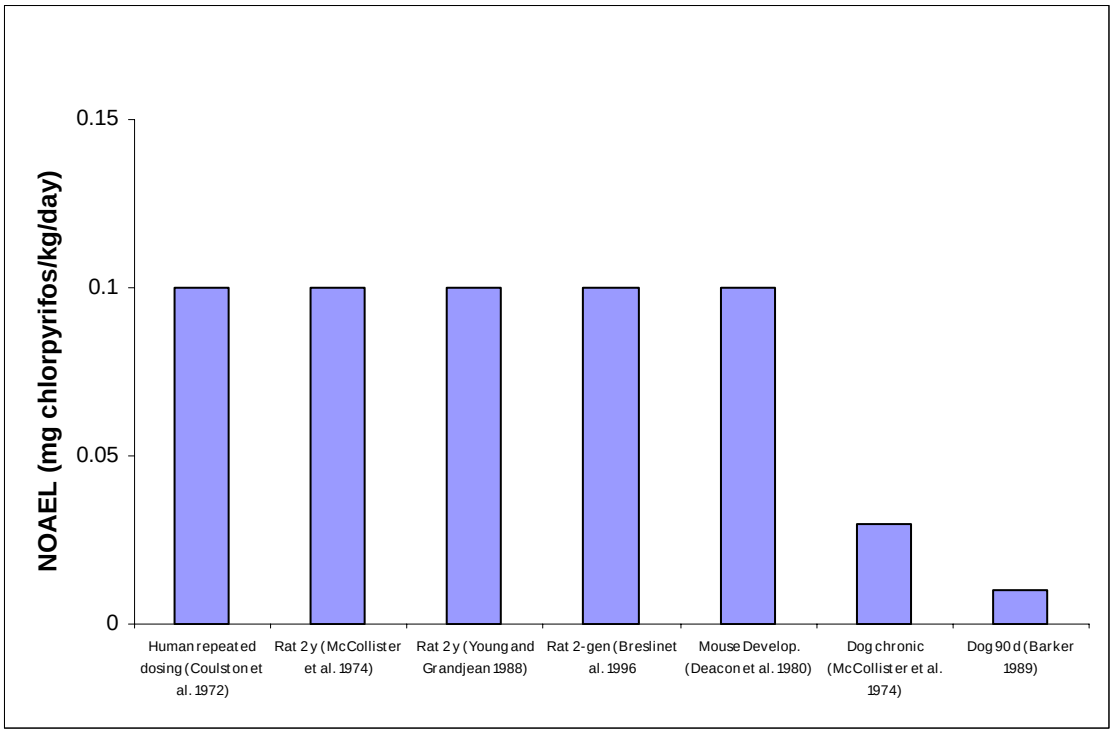
944 Figure 1



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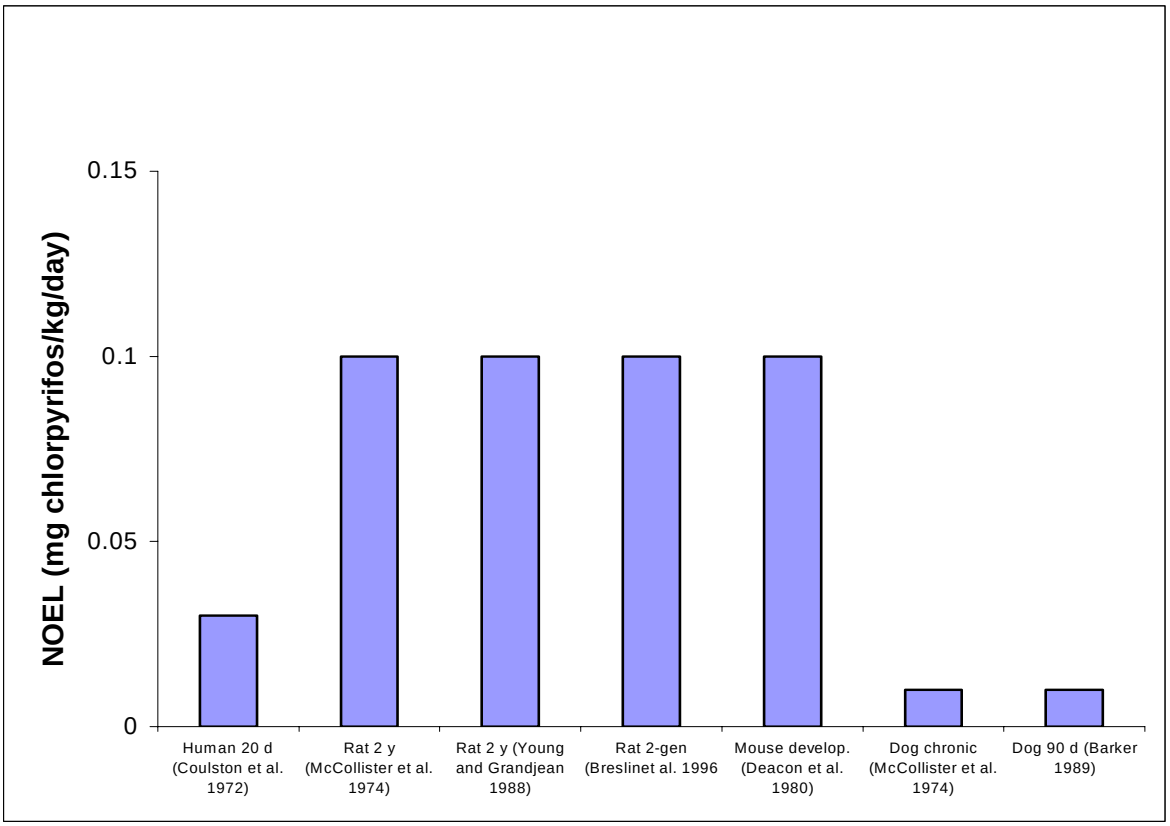

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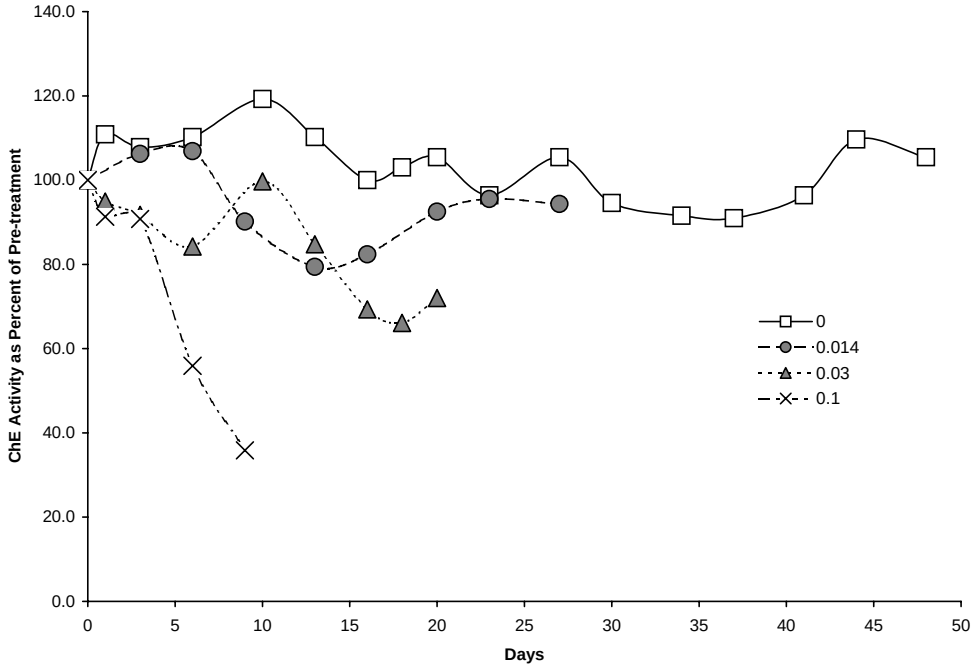

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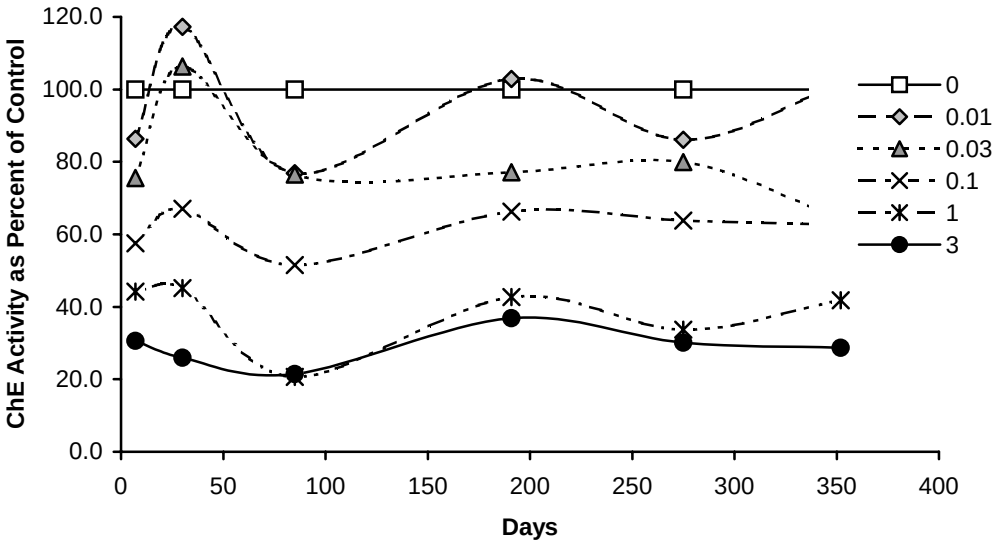

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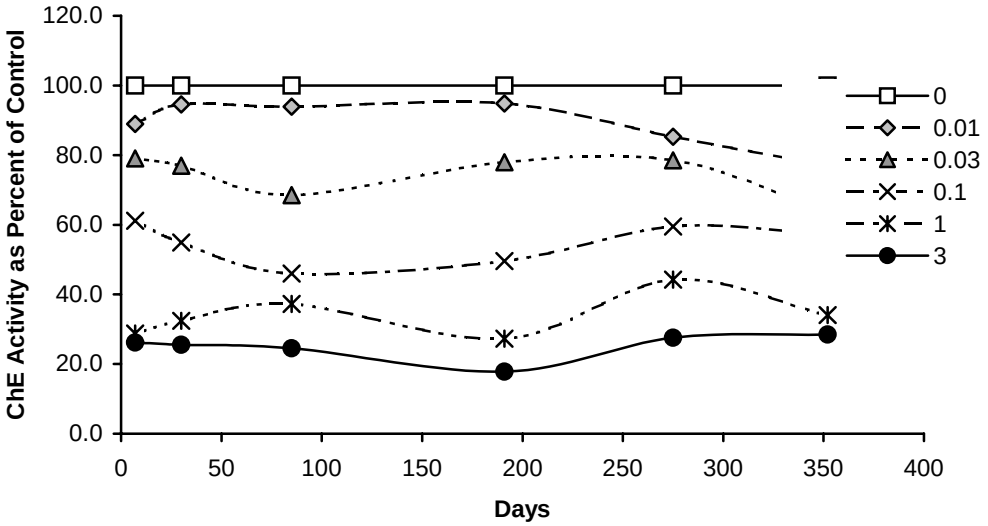
979 Figure 5



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981 Figure 6

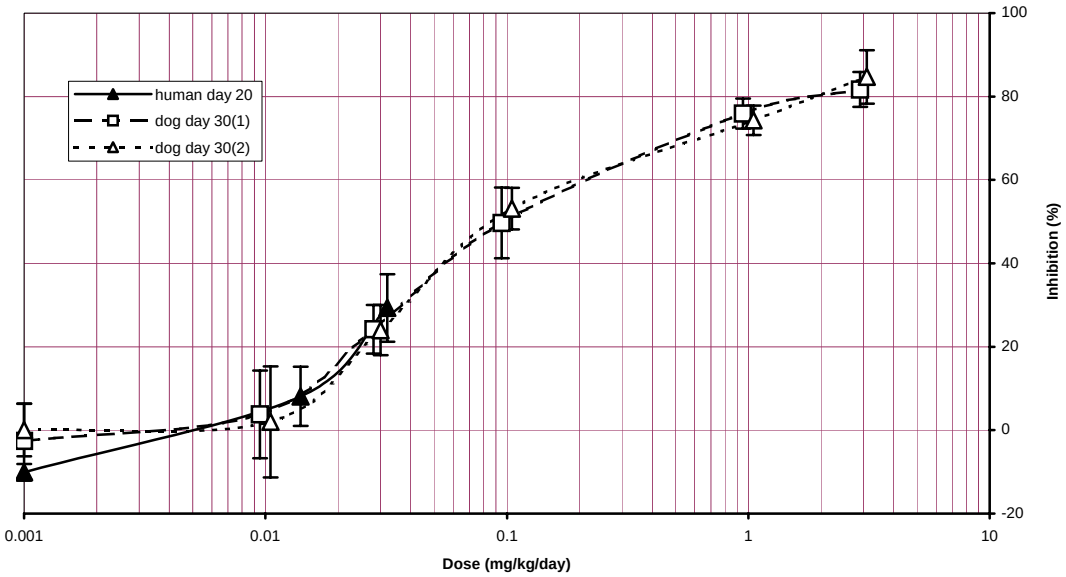

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Figure 1: NOAEL of chlorpyrifos-induced RBC ChE inhibition in different animal species. NOAEL = no-observed-adverse-effect-level in mg/kg/day.

Figure 2: NOEL of chlorpyrifos-induced plasma ChE inhibition in different animal species. NOEL = no-observed-effect-level in mg/kg/day.

Figure 3: Human plasma ChE activity following repeated exposure to chlorpyrifos. Plasma ChE activity was expressed as percent of the control value. Each line represents response at a treated dose (mg/kg/day). Data were obtained from Coulston *et al*. (1972).

Figure 4: Female dog plasma ChE inhibition in one-year study. Plasma ChE activity is presented as percent of the control level. Each line represents the responses in a particular dose group (mg/kg/day). Data are obtained from McCollister *et al*. (1974).

Figure 5: Male dog plasma ChE inhibition in one-year study. Plasma ChE activity is presented as percent of the control level. Each line represents the responses in a particular dose group (mg/kg/day). Data are obtained from McCollister *et al*. (1974).

Figure 6. Plasma BuChE Inhibition in both Dogs and Humans for either 20 or 30 days. Data were adjusted to reflect only BuChE. Human and dog data were obtained from Coulston *et al*. (1972) and McCollister *et al*. (1974), respectively. Detailed the BuChE inhibition analysis is available at www.tera.org.