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Lower Birth Weight as A Critical Effect of Chlorpyrifos: A Comparison of Human and Animal Data

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Abstract

Chlorpyrifos is an irreversible inhibitor of cholinesterase (ChE), and inhibition of ChE is believed to be the most sensitive effect in all animal species evaluated and in humans. Recent epidemiology studies reported associations between umbilical cord plasma chlorpyrifos levels and fetal birth weight decreases among minority women living in New York City during pregnancy. These associations raise questions whether impaired fetal development is the critical effect rather than the inhibition of ChE as is currently believed so. We analyze the available information from epidemiology studies and animal studies in order to identify the relative sensitivity of decreased birth weight and inhibition of ChE from exposure to chlorpyrifos. We find that the positive associations from some epidemiology studies are different from other epidemiology investigations. Moreover, a direct comparison of experimental animal neonatal information shows that cholinesterase inhibition is a more sensitive indicator of adverse effect than reduced body weight, and that neonates are equally, or perhaps less sensitive to cholinesterase inhibition than their maternal parent. Based on a review of 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 any fetal developmental effect. Moreover, the critical effect for chlorpyrifos still appears to be cholinesterase inhibition.

Introduction

Chlorpyrifos is one of the most widely used organophosphate insecticides in the U.S. This chemical is moderately toxic following acute oral, dermal, and inhalation exposures. It is an irreversible inhibitor of cholinesterase (ChE) including acetylcholine esterase (AChE), and inhibition of AChE in the central and peripheral nervous systems causes accumulation of acetylcholine, a neurotransmitter, which in turn results in neurotoxicity in animals and humans. Inhibition of ChE is believed to be the most sensitive response in all animal species evaluated and in humans, regardless of route or duration of exposure (U.S. EPA, 1999).

In a recent 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. 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 raises a question as to whether impaired fetal development could be the critical effect rather than the inhibition of AChE as has been believed so far.

In this paper, we analyze the available information from additional epidemiology studies and animal studies in order to identify the relative sensitivity of decreased birth weight and inhibition of ChE from exposure to chlorpyrifos. We also discuss whether decreased birth weight or ChE inhibition is the critical effect of chlorpyrifos exposure.

Methods

A key step in the hazard identification for a noncancer assessment is evaluation of all available data and identification of the critical effect. This includes characterization of the quality of the evidence from human and animal studies, and other supportive information. Hill (1965) provided criteria for evaluating whether a causal relationship has been established in an epidemiology study, and in the overall epidemiology database. Hill considered that the strength of the association could be enhanced when consistent results are obtained by different investigators under a variety of circumstances, or the association is consistent with what is known about the chemical's effects and mechanism based on clinical or animal studies. As noted by U.S. EPA (1994), these same criteria apply in an evaluation of the weight of evidence for the entire database or when applied in the evaluation of experimental animal data (Haber et al., 2001). In addition to the general principles, the strength of the overall evidence is enhanced if species-specific differences in sensitivity to a chemical, if observed, are understood.

The use of ethically-derived human data to protect the public health is recommended (e.g., National Academy of Sciences, 2004). Whenever possible, human data are preferred to animal data in human risk assessment (e.g., Barnes and Dourson, 1988; EPA, 2002). In the case of conflicting interpretations of human data, however, experimental animal work can be used to explore suggested modes of chemical action and critical effects, and can often resolve apparent contradictions. The use of experimental animal information in environmental risk assessment is routine and extensive. Such experimental animal investigation can also lead to more definitive work

in either humans or experimental animals if sufficient uncertainty exists in a chemical's safe dose.

In the present analysis, we evaluated three recently published epidemiology studies in a side-by-side comparison and integrated the results into an analysis of prior human clinical and experimental animal work in an attempt to determine whether reduced fetal body weight is a more sensitive indicator of effect than cholinesterase inhibition, the current critical effect for chlorpyrifos. With the animal data, we limited ourselves to developmental and reproductive studies that evaluated fetal growth, as well as studies that investigated ChE inhibition, as these effects have been used to determine the safe dose of chlorpyrifos by multiple agencies (e.g., ATSDR, 1997; U.S. EPA, 2000; U.K. ACP, 2003) and independent parties (e.g., Clegg and van Gemert, 1999; van Gemert et al. 2001). These animal studies also provide valuable information for evaluation of the possible causes of fetal growth effects due to chlorpyrifos exposure. The analysis provided here on the available epidemiology work on chlorpyrifos is not meant to be exhaustive. To accomplish this task, we followed the U.S. EPA Reference Dose (RfD) method (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA 2002), but focused on the hazard assessment step of the risk assessment paradigm.

Results and Discussion

Comparison of Recent Epidemiology Studies on Fetal Developmental Effects

Whyatt *et al*. (2004) investigated an association between chlorpyrifos exposure among minority women in New York City during pregnancy and fetal development. The authors found a significantly inversed association between umbilical cord plasma

chlorpyrifos levels and fetal birth weight and birth length. However, no association was found between maternal personal air insecticide levels and birth weight, length or head circumference.

Two other research groups (Berkowitz *et al*. 2004; Eskenazi *et al*. 2004) also independently conducted similar epidemiology studies to investigate the association between exposure to chlorpyrifos and fetal development, but used different exposure biomarkers than those used by Whyatt *et al.* (2004). Table 1 provides summary information on the three epidemiology studies (Whyatt *et al*. 2004; Eskenazi *et al*. 2004; Berkowitz *et al*. 2004) in terms of population background, study population selection, biological samples, exposure measurement, and study results.

The Whyatt *et al*. (2004) and Berkowitz *et al*. (2004) studies evaluated populations living in the Harlem area of New York City during the same period of time (1998–2003). These populations were exposed to chlorpyrifos through residential use of the pesticide. Whyatt *et al*. (2004) focused their study on a minority population that was 58% African American and 42% Dominican in the area north of Central Park, while Berkowitz *et al*. (2004) investigated a population that was 50% Hispanic, 21% white, and 28% African American located in the Mount Sinai area. The two populations reported comparable pesticide usages during pregnancy: 71.5% of those in the Berkowitz *et al*. (2004) study and 84-86% in the Whyatt *et al*. (2004) study.

Whyatt *et al*. (2004) reported an association between chlorpyrifos exposure in their highest dose group and decreased birth weight ($P = 0.03$), and birth length ($P=0.04$). These authors reported chlorpyrifos level of 2.5 pg/g in cord blood (geometric mean prior to 1/1/2001) as a direct measure of fetal exposure. This exposure index seems ideal from

a risk assessment perspective, but the cited exposures based on personal air monitoring were admittedly "well below the U.S. EPA reference dose" for chlorpyrifos, leading the authors to encourage additional research to confirm or refute their findings. A recent published review paper (Brent et al. 2004) also raised some concerns about this observation because of very low levels of chlorpyrifos in the serum and small differences in growth indices observed in the study.

Berkowitz *et al*. (2004) found no associations between maternal urinary 3,5,6-trichloro-2-pyridinol (TCPy, a primary chlorpyrifos metabolite) and fetal growth indices including birth weight, birth length, and head circumference. However, they did find an inverse relationship between head circumference and detected TCPy concentrations in mothers with low paraoxonase (PON1) activity, a known detoxification enzyme for chlorpyrifos. In this study, maternal urinary TCPy level varied from 1.6 to 32.5 μg/L (interquartile range). Although it is not known whether chlorpyrifos can cause adverse effect on fetal development through TCPy, the increase in TCPy in the mother's urine should correlate with an elevated exposure to its parent compound, chlorpyrifos. Should chlorpyrifos affect fetal development, a correlation between chlorpyrifos' metabolite and fetal measurements of growth such as birth weight might also be expected based on the work of Whyatt *et al*. (2004). However, such an association was not seen in the work of Berkowitz *et al*. (2004), possibly due to the different measurements used. Urinary TCPy appears to be a reasonable dose surrogate for the fetus, although perhaps not as good as chlorpyrifos cord blood measurements.

Eskenazi *et al*. (2004) conducted a cohort study in a different population from those used by Whyatt *et al*. and Berkowitz *et al*. The subjects in Eskenazi *et al*. study

came from a low-income population living in Salinas Valley, CA, and about 84% of people in the population were born in Mexico. Approximately 28% of the women had worked in agricultural fields during their pregnancies, and another 14% had worked at other jobs in agriculture, including packing shed, nursery, and greenhouse work. In this study, TCPy was measured as an estimate for the exposure to chlorpyrifos. TCPy concentrations in the urine ranged from 0.2 μg/L to 56.1 μg/L with a median concentration of 3.3 μg/L. This concentration is in the same range as that measured in the population living in the Mt. Sinai area (Berkowitz *et al*. 2004). The authors found no significant associations between urinary TCPy and the aforementioned fetal growth indices. In addition, there was no association between ChE activity in either maternal or cord blood samples and fetal growth indices. However, an association was observed between decreases in gestational duration without clinical impact and a measure of *in utero* pesticide exposure, umbilical cord whole blood ChE. The lack of findings of fetal growth indices could be due to two reasons. The first is that the population exposure level was too low to cause any ChE inhibition, and perhaps too low to cause any change in fetal growth indicies, since blood ChE inhibition in animal developmental studies (see discussion below) indicates that ChE inhibition is more sensitive than changes in fetal growth indices. Another possibility is that there is no association between ChE inhibition and fetal growth indices. In either of these events, at the level of chlorpyrifos exposure experienced by the population living in Salinas Valley, the overall results are in contrast to those seen in the Whyatt *et al*. study.

Sensitivity of ChE Inhibition and Fetal Growth in Experimental Animals

Several multi-generation studies of reproductive and developmental toxicity in rats, which investigated ChE inhibition and fetal development, have been extensively reviewed (IPCS 1999; Schardein and Scialli, 1999; U.K. ACP 2003) (see Table 2). Results from these studies indicate that generally, statistically significant decrease in fetal birth weight starts to be seen at 5.0 mg/kg/day, with doses of ≥5 mg/kg/day causing maternal toxicity such as decreased body weight during lactation and brain AChE inhibition. In contrast to these general findings, a two-generation study conducted by James *et al*. (1988) (see Table 2) reported a statistically significant decrease in pup mean weight in F_1 pups at ≥0.5 mg/kg/day accompanied by statistically significant increases in the F_1 litter size and litter weight. However, there was no consistent relationship in these changes with dose, and the changes were generally only slightly different from the controls. In reviewing this study, the Joint Meetings of the FAO (Food and Agriculture Organization) Panel of Experts on Pesticide Residues in Food and the Environment and the WHO (World Health Organization) Core Assessment Group (IPCS, 1999) noted that the decrease in pup weight observed by James *et al*. was probably related to the increase in litter size, and not treatment related. The increases in litter size and litter weight seen in this study (James *et al*., 1988) were not observed in other studies including those that reported fetal/pup weight changes at dose levels ≥5 mg/kg/day. Therefore, the reported decrease in pup mean weight at ≥0.5 mg/kg/day is more likely due to increased litter size instead of chemical toxicity. Other investigators have also reported a significant but non-dose-dependent increase in fetal body weight at ≥3 mg/kg/day (Oulette *et al*., 1983) and a slight but statistically significant <u>increase</u> in F_1 pup mean weight at 15 mg/kg/day (Rubin

et al., 1987). No significant changes in litter size were reported in these studies either. Since the increased pup body weight were not repeated by most of the other developmental studies, the increase was considered to be of no toxicological importance. Based on the weight of evidence from animal studies, the threshold dose for causing decrease in fetal body weight is around 5 mg/kg/day. Given the significant maternal toxicity observed at the same treatment dose, the observed effect on fetal birth weight might be a secondary effect to the maternal toxicity caused by chlorpyrifos treatment.

It, therefore, appears that the decreased fetal body weight is not the most sensitive effect observed in these developmental studies. From Table 2, both maternal plasma and RBC ChE inhibitions occur at doses (ranging from 0.3 mg/kg/day to 1.0 mg/kg/day) lower than those causing effect on fetal birth weight (5.0 mg/kg/day) in the same studies. Therefore, ChE inhibition in either maternal blood plasma or RBCs is likely to be more sensitive (at least > 5-fold) to chlorpyrifos treatment than the effect on fetal birth weight.

Other *in vivo* studies on the effects of chlorpyrifos exposure through parenteral route during early and late gestation also supported the findings from aforementioned oral treatment studies. Chlorpyrifos subcutaneously administered to rats during pregnancy [gestation day (GD) 17-20] evoked fetal body weight changes at ≥10 mg/kg/day (compared to maternal growth impairment at ≥5 mg/kg/day; Garcia *et al*., 2002; Qiao *et al*., 2002) while in the same studies, chlorpyrifos at ≥1 mg/kg/day significantly inhibits fetal brain AChE activity compared to ≥2 mg/kg/day in pups. It is worth noting that AChE inhibition in the brain is usually less sensitive to chlorpyrifos treatment than the inhibition of ChEs in the blood plasma and RBCs (U.S. EPA, 1999), and there are no clinical signs of cholinergic toxicity below 70% inhibition of brain cholinesterase (Clegg

and van Gemert, 1999). Cognitive or behavioral defects are not observed until substantial brain cholinesterase inhibition occurs. Therefore, inhibition of ChE either in the blood plasma and RBCs, or brain should be considered a precursor to the adverse neurotoxicity effect.

Comparison of Chlorpyrifos Internal Dose Between Human and Animal Studies

Another way to evaluate the positive observation reported by Whyatt et al. (2004) is to examine the internal doses experienced by those pregnant women living in New York City, and to compare the estimated internal doses to animal internal doses that caused adverse response. Therefore, the internal dose can be used as a biomarker in evaluation of the relative exposure levels and possible corresponding outcomes.

Mattsson *et al*. (1998, 2000) tested ChE activity in five different organs in rat dams and their fetuses or pups at different time points, for three different doses and a control group. A unique aspect of the Mattsson *et al*. (1998, 2000) work is that levels of chlorpyrifos and TCPy in the blood of both the dam and corresponding fetus or pup were measured. Thus, if we consider that rats and humans are roughly similar in RBC ChE inhibition response and within a 3-fold difference in plasma inhibition as discussed below, and if we further assume a similar sensitivity between adult and infant humans as shown between adult and infant rats as described by Zheng *et* al. (2000), we can roughly compare the human exposure doses as measured by chlorpyrifos concentration in the cord blood in Whyatt *et al*. (2004) to the blood concentration in rat fetuses (Mattsson *et al*., 2000) to estimate the expected levels of ChE inhibition in the exposed human populations.

In rats (Mattsson *et al*., 2000), daily chlorpyrifos treatment (0, 0.3, 1.0 or 5.0 mg/kg/day) during pregnancy resulted in blood chlorpyrifos concentrations of 0, 0, 3, and 109 ng/g, respectively, in dams (analyzed on GD 20), and corresponding concentrations of 0, 0, 1, and 46 ng/g in fetuses (see Table 3). The corresponding plasma ChE activities were 100%, 67%, 39% and 12% of control ChE level in the dams, and 100%, 104%, 96% and 15% in the fetuses (see Table 4). Similarly, blood chlorpyrifos concentrations and corresponding ChE levels in the plasma and RBCs up to postnatal day 22 can also be obtained (Tables 3&4). Combining these data in Tables 3&4 into Table 5 allows us to plot *in vivo* chlorpyrifos concentrations in the blood against corresponding ChE activities in the plasma (Figure 1) and RBCs (Figure 2) in dams and corresponding fetuses or pups. This data summary was also presented in Scheuplein *et al*. (2002). There is a clear dose-response relationship between the internal doses as measured by chlorpyrifos blood concentration and ChE activities in the blood (see Figures 1 and 2). The no-observable-effect levels (NOELs) for fetus and pups for both plasma and RBC ChE inhibition are 1 ng chlorpyrifos /g blood in this analysis. The NOEL for dams is determinable by a BMD analysis (not shown here), but apparently lower.

Whyatt *et al*. (2004) reported that in their cohort in New York City, the chlorpyrifos concentration in umbilical cord blood was 2.5 pg/g prior to 1/1/2001 and 0.6 pg/g after 1/1/2001 (Fig. 1 in Whyatt *et al*. 2004). This internal dose of 2.5 pg chlorpyrifos /g in human cord blood is about 1/400 of the 1 ng chlorpyrifos /g in the blood from rat fetuses, which failed to elicit any ChE inhibition (plasma or RBCs) in rat fetuses (Mattsson *et al*. 2000).

In addition, humans are comparable to animals in terms of sensitivity to chlorpyrifos-induced ChE inhibition and its related toxicity. In recent assessments (ATSDR, 1997; U.S. EPA, 2000; U.K. ACP, 2003), chlorpyrifos has been evaluated for toxicity after oral administration in humans, rats, mice, and dogs. In all experimental animal species, the most sensitive response is inhibition of plasma, RBC, and brain ChE. Following chronic exposure, dogs appear to be the most sensitive species for ChE inhibition and systemic effects and are more sensitive than humans to the effect of chlorpyrifos in terms of ChE inhibition in plasma or RBC (McCollister et al., 1974; Coulston et al., 1972). Humans also appear 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 (Young and Grandjean, 1988; Breslin et al., 1996; Deacon et al., 1980).

Thus, based on 400-fold lower exposure level than animal NOEL in the human population living in New York City and a comparable response to chlorpyrifos between humans and animals, it is unlikely that the exposure level encountered by the human population in Whyatt *et al* (2004) study would be associated with ChE inhibition in human fetuses and/or newborns. In fact, lack of ChE inhibition in either maternal or cord blood is evident in the population (Eskenazi et al., 2004) living in Salinas Valley, CA, which exposed to similar external dose of chlorpyrifos as those living in New York City (Berkowitz et al., 2004, Whyatt et al., 2004). Since our analysis of animal developmental studies indicates that ChE inhibition is a more sensitive response to chlorpyrifos exposure than fetal developmental effects, the weight of evidence does not support that the exposure to chlorpyrifos encountered by the human population living in New York City would cause decreased fetal birth weight or body length.

Conclusion

The integration of information from epidemiology and experimental animal studies is needed prior to the development of any comprehensive environmental decision. In the case of chlorpyrifos, for example, it may be inappropriate to use the results of Whyatt *et al*. (2004) study by themselves to make statements about risk at low levels. Likewise, it may be inappropriate to use the results of experimental animal studies by themselves to make such statements. A reasonable course of action, and indeed one that is followed by health agencies around the world, is to consider such human and experimental animal data together in any development of safe dose (e.g., Dourson *et al*. 2001).

Whyatt *et al*. (2004) reported a troubling association between umbilical cord plasma chlorpyrifos level and lower fetal birth weight among women living in New York City. This finding raises a concern about the possible impaired fetal development following exposure to chorpyrifos during pregnancy. 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. In our initial attempt to evaluate these data, we found that the positive association from Whyatt *et al*. (2004) study is inconsistent with the results of two other epidemiology investigations or with the determination of critical effect for safe dose assessment by a number of groups. Moreover, a direct comparison of experimental animal neonatal information shows that cholinesterase inhibition is a more sensitive

indicator of effect than reduced body weight, and that neonates are equally, or perhaps less sensitive to cholinesterase inhibition than their maternal parent. Based on comparison of human cord blood chlorpyrifos concentration and blood chlorpyrifos concentrations that in animals caused dose-response reaction, it is unlikely that the exposure level encountered by the population reported in Whyatt *et al* (2004) study would be associated with ChE inhibition in human fetuses and/or newborns, a more sensitive endpoint than fetal developmental effect.

However, like Whyatt et al (2004) we invite additional review of these epidemiology studies and experimental animal work to further investigate the causal relation between exposure to chlorpyrifos or other coexisting compounds and fetal developmental effects. Risk assessment scientists need a strong foundation for establishing an updated risk value for chlorpyrifos.

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Table 1. Comparison of Epidemiology Studies

	Berkowitz *et al*. 2004	**Whyatt *et al*. 2004**	**Eskenazi *et al*. 2004**
Sample size	404	314	488
Residential area	Mount Sinai (east Harlem), New York City	Harlem (north of central park), New York City	Salinas Valley, CA
Ethnicity	Hispanics (predominantly Puerto Rican), African, whites	African, and Dominican	Majority of Hispanic
White	21%	0	N/A
African	27.7%	58%	N/A
Hispanic	49.8%	42%	84% born in Mexco
other	1.5%	0	N/A
Sample	Maternal blood, cord blood, and urine	Maternal blood, cord blood	Maternal blood, cord blood, and urine
Reported pesticide use during pregnancy	71.5%	84-85.6%	42% worked either in the fields or other job in agriculture
Measurement in urine	TCPy	N/A	Dialkyl phosphate metabolites, pesticide specific metabolites (TCPy)
TCPy in urine	7.6 (interquartile range: 1.6-32.5) μg/L	N/A	3.3 (0.2-56.1) μg/L
Measurement in maternal blood and cord blood	PON1 activity	chlorpyrifos in the blood	ChE in whole blood, BChE in the plasma
ChE in blood	N/A	N/A	Maternal: 5.1 (0.7-10.2) μmol/min/ml Cord: 3.8 (1.7-6.1) μmol/min/ml
BChE in plasma	N/A	N/A	Maternal: 1.4 (0.6-3.9) μmol/min/ml; Cord: 1.2 (0.6-2.7) μmol/min/ml.
Finding	There is no association between TCPy and fetal growth indices; Maternal PON1 activity is associated with head circumference when urine TCPy>LCD	There is an association between cord blood chlorpyrifos concentration and fetal birth weight as well as birth length, but not head circumference. However, there is no association between chlorpyrifos in maternal personal air samples and fetal growth indices;	Diethyl phosphates and TCPy were not associated with fetal body weight changes; there was no association between maternal or fetal blood or plasma ChE inhibition and fetal birth weight, length and head circumference.

TCPy: 3,5,6-Trichloro-2-pyridinol
ChE: Cholinesterase
BChE: Butyryl cholinesterase

Table 2. Comparison of Animal Developmental and Reproductive Studies

Reference	Study type	Species	Test doses (mg/kg/day)	Fetal/pup body weight changes	Plasma ChE inhibition	RBC ChE inhibition	Brain ChE inhibition
Ouelette *et al*. (1983)	Developmental study	Fischer 344 rat	0, 0.1, 3 or 15	≥3.0 mg/kg/day caused significant increase in fetal body weight; not dose-dependent	≥3.0 mg/kg/day caused inhibition in dams	≥3.0 mg/kg/day caused inhibition in dams	Not analyzed
Rubin *et al*. (1987)	Developmental study	Sprague-Dawley rat	0, 0.5, 2.5 or 15	15 mg/kg/day caused slight but statistically significant increase in mean fetal weight; not considered to be of toxicological significance	≥0.5 mg/kg/day caused inhibition in dams (dose-related) on GD 15.	Not analyzed	Not analyzed
James *et al*. (1988)	Two-generation reproduction study	Sprague-Dawley rat	F_0: 0.1-0.2, 0.5-0.9 or 2.5-4.5 (males); 0.1-0.2, 0.6-0.9 or 2.9-4.6 (females) F_1: 0.1-0.3, 0.7-1.6 or 3.3-8.1 (males); 0.2-0.3, 0.8-1.6 or 4.0-8.1 (females)	≥0.5 caused statistically significant decrease in F_1 but not F_2 pup weight. It is probably related to increased litter size. In addition, these changes were only slightly different from those in the controls and there was no consistent relationship with dose.	N/A	N/A	N/A
Breslin *et al*. (1991)	Two-generation reproduction study	Sprague-Dawley rat	0, 0.1, 1.0 or 5.0	5 mg/kg/day caused statistically significant decrease in birth weight (in F_1 pups, but not in F_2 pups); F_0 maternal toxicity observed at this dose	≥1.0 mg/kg/day caused ChE inhibition in F_0 and F_1 adults	≥1.0 mg/kg/day caused RBC ChE inhibition in F_0 and F_1 adults	5 mg/kg/day caused brain ChE inhibition in F_0 and F_1 adults
Breslin *et al*. (1996)	Two-generation reproduction studies	SD rats	0, 0.1, 1 or 5 (reproduction)	≥5 mg/kg/day caused slightly, but not significantly, decreased birth weight in F_1 & F_2 litters	≥1 mg/kg/day caused ChE inhibition in P_1 and P_2 rats.	≥1 mg/kg/day caused RBC ChE inhibition in P_1 and P_2 rats.	5 mg/kg/day caused inhibition in P_1 and P_2 rats
Hoberman (1998)	Developmental study	SD rat	0, 0.3, 1 or 5	5 mg/kg/day caused reduction in birth weight; maternal toxicity observed at the same dose	≥0.3 mg/kg/day caused inhibition in dams	≥0.3 mg/kg/day caused inhibition in dams	≥1 mg/kg/day caused inhibition in dams
Maurissen *et al*. (2000)	Developmental study	SD rats	0, 0.3, 1 or 5	5 mg/kg/day caused significant decrease in pup body weight	≥ 0.3 mg/kg/day caused inhibition in dams	≥ 0.3 mg/kg/day caused inhibition in dams	≥1 mg/kg/day caused inhibition in dams

N/A: Not analyzed

Table 3. Comparison of Blood Chlorpyrifos (CPF) in Dams and Their Corresponding Fetuses and Pups. Data are from Mattsson et al. (1998, 2000).

CPF Dose (mg/kg-day)	Blood CPF Concentration (ng/g of blood)							
	GD 20		PND 1		PND 5		PND 11	
	Maternal	Fetus	Maternal	Pup	Maternal	Pup	Maternal	Pup
0.0	0	0	0	0	0	0	0	0
0.3	0	0	0	0	0	0	0	0
1.0	3	1	0	0	0	0	0	0
5.0	109	46	15	12	15	0	0	0

GD = Gestation Day
PND = Post Natal Day

Table 4. Comparison of Cholinesterase Activity as a Funciton of Chlorpyrifos Dose (mg/kg-day) and Time of Maternal Plasma and RBC with Their Corresponding Fetuses and Pups. Data are from Mattsson et al., 1998.

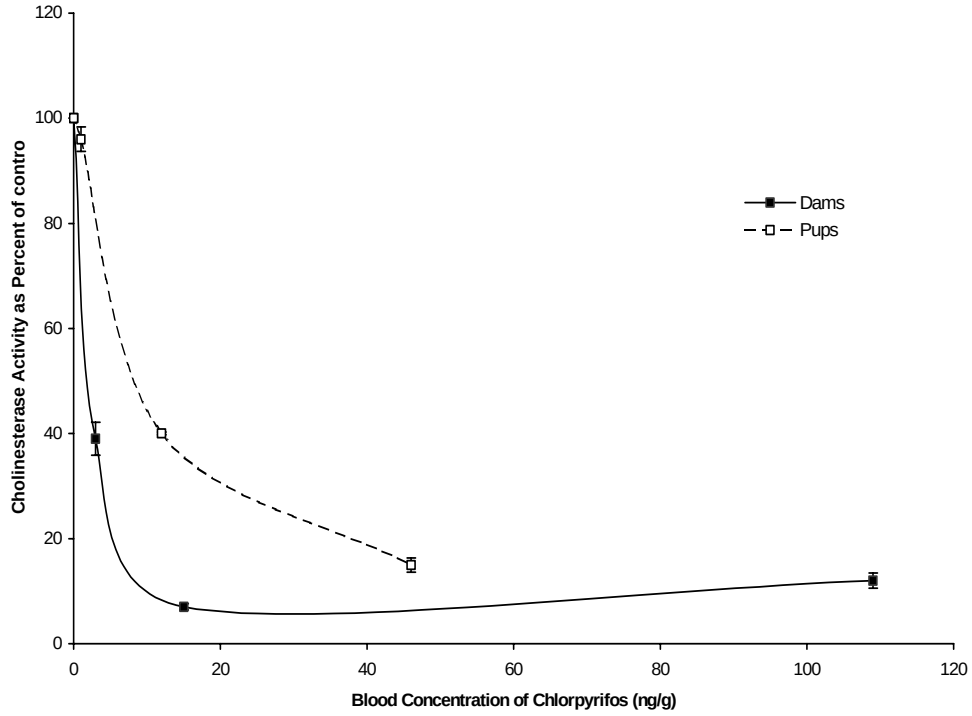
Dose	**GD 20**				**PND 1**				**PND 5**				**PND 11**				**PND 22**			
	Maternal		Fetal		Maternal		Fetal		Maternal		Fetal		Maternal		Fetal		Maternal		Fetal	
	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC
0.0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
0.3	67	74	104	102	48	61	98	111	68	89	105	104	84	76	93	101	117	93	96	102
1.0	39	18	96	106	23	13	94	101	31	16	108	105	66	23	96	97	132	67	96	110
5.0	12	5	15	8	6	1	40	15	8	3	82	57	49	7	91	86	120	54	96	104

Table 5. Comparison of Cholinesterase Inhibition in the plasma and RBC as a Function of Blood Concentrations (ng/g) of Chlorpyrifos (CPF) at All Times in Dams and Their Corresponding Fetuses and Pups. Cholinesterase Values Are in Percent of Control.

Blood Concentration of CPF ng/g	Plasma		RBC	
	Dams	Pups	Dams	Pups
0	100	100	100	100
1		96		106
3	39		18	
12		40		15
15*	6		1	
15*	8		3	
46		15		8
109	12		5	

* Plotted in Figures 1&2 as one point.

Figure 1

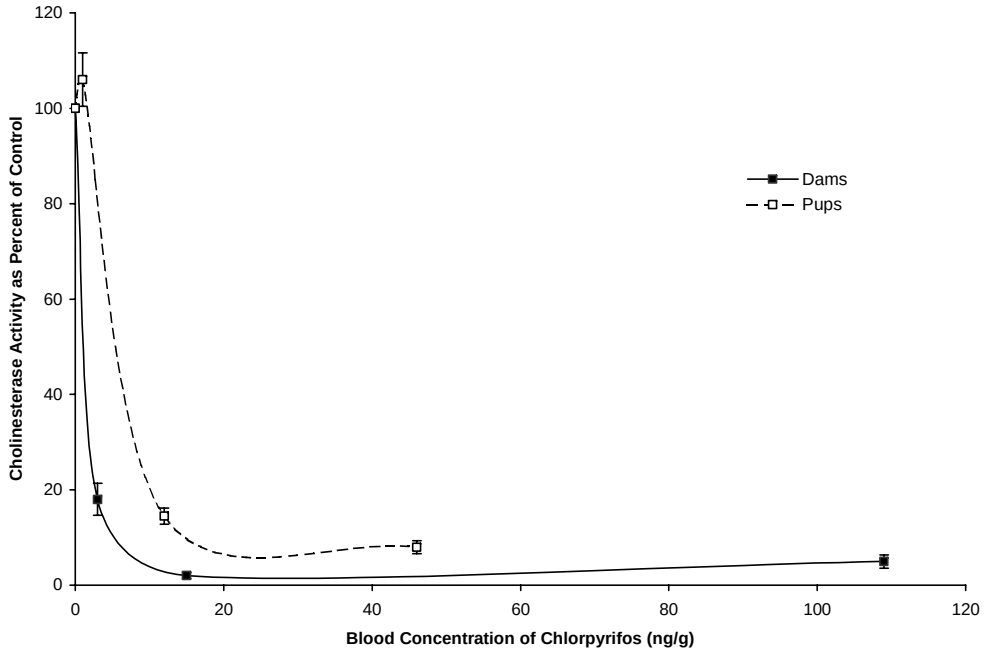

Cholinesterase Activity as Percent of contro
Blood Concentration of Chlorpyrifos (ng/g)
Dams
Pups
0
20
40
60
80
100
120

38

39

40

40 Figure 2



41

42

Figure legend:

Figure 1: Comparison of plasma ChE activity in rat dams and their corresponding fetuses or pups at all time points. Plasma ChE activity is presented as percent of the control level. Error bars indicate sample standard error. Solid line: Dams treated with CPF from GD6 to PND10. Dash line: fetuses or pups from treated dams. Data are obtained from Mattsson et al. (1998, 2000).

Figure 2:

Comparison of RBC ChE activity in rat dams and their corresponding fetuses or pups at all time points. RBC ChE activity is presented as percent of the control level. Error bars indicate sample standard error. Solid line: Dams treated with CPF from GD6 to PND10. Dash line: fetuses or pups from treated dams. Data are obtained from Mattsson et al. (1998, 2000).