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Data Evaluation Record

Study Type: Special *Non-Guideline* Assessment for RBC Cholinesterase in Humans.

DP Barcode: D255538
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Submission No.: S561108
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Test Material: Dursban[®] 99.8% Chlorpyrifos (Lot No.: MM9300503-17)

Citations: Kisicki, J.C., Seip, C.W. and Combs, M.L., 1999, "A Rising Dose Toxicology Study to Determine the No-Observable-Effect-Levels (NOEL) for Erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels", MDC Harris Laboratory, Lincoln Nebraska, Study No.: 21438 (for the Harris Project) and DR K-0044793-284 (for DOW AgroSciences), April 19, 1999, MRID No.: 44811002.

and

Brzak, K.A. "A Rising Dose Toxicology Study to Determine the No-Observable-Effect Levels (NOEL) for Erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels - Part B (Pharmacokinetic paraoxonase data)". Toxicology and Environmental Research and Consulting, DOW, Study No.: 981176, June 5, 2000. MRID No.: 45144101.

Sponsor: DOW AgroSciences.

Executive Summary:

In this special study (1999, MRID No.: 44811002) to assess for possible effects on human red blood cell (RBC) acetylcholinesterase (AChE), subjects were in the first phase were dosed orally as control, 0.5 or 1.0 mg/kg of chlorpyrifos (99.8%) in a gelatin capsule and when it was determined that there were no reactions in these subjects, a second phase of 6/sex/group were dosed as control and 2.0 mg/kg for a total of five groups of 6/sex. The subjects were evaluated by physical examinations to test for blood pressure and any reactions to treatment, clinical chemistry and urinalysis. Blood was assessed at -10, zero, 2, 4, 8, 12, 24, 36, 48, 72, 96, 144 and 168 hours for RBC AChE. Plasma ChE was not assessed. In addition to the clinical aspects of this study, pharmacokinetic data on chlorpyrifos, chlorpyrifos oxon and the principle metabolite 3,5,6-trichloro-2-pyridinol (TCP) and assessment of paraoxonase and chlorpyrifos oxonase (CPOase) were reported (MRID No.: 45144101).

Systemic Effects. There were no clinical reactions or changes in clinical chemistry parameters related to treatment. However, it could not be determined if one subject (#56) in the 2 mg/kg dose group who unexpectedly left the study after 48 hours developed delayed symptoms in response to chlorpyrifos after leaving the study or if the reason the person left the study was because of reactions to treatment.

Pharmacokinetic Data. Chlorpyrifos was detected in the serum in only a few subjects and in one subject (#56) a peak concentration of 18 ng/gm at 8 hours. This same subject also had the highest levels of serum and urinary TCP accumulated up to 48 hours when she left the study. The serum chlorpyrifos and serum and urinary TCP data all suggested that this subject absorbed more of her dose than the other subjects on the study. CPOase for this subject was typical of the other subjects.

RBC AChE. Zero time (predosing) baseline RBC AChE was from 8143 ± 982 ($\pm 12\%$) to 9572 ± 539 ($\pm 6\%$) U/L for males and 8576 ± 556 ($\pm 6\%$) and 9165 ± 709 ($\pm 8\%$) U/L for females for each of the five groups in this study indicating good precision for the baseline data. These RBC AChE values were within the expected range for humans. Only one individual had a decrease in RBC AChE that implied inhibition by chlorpyrifos as indicated by being *consistently decreased* at the critical hours post dosing. For this subject (#56, a female dosed with 2 mg/kg), inhibition of RBC AChE was obvious starting at 8 (23% ↓), 12 (maximum 28% ↓), 24 (26% ↓), 36 (19% ↓) and 48 (21% ↓) hours. There were no obvious associated symptoms reported. A transient report of numbness in the upper arms in this subject occurred at about 2 hours or well before AChE inhibition and significant levels of TCP in the serum.

This study has two problems that would contribute to assigning an unacceptable classification to this study. *The first is that no assessments of plasma cholinesterase were made.* The second is that the only subject showing an apparent effect of treatment (manifested by up to 28% decrease in RBC AChE) did not complete the study and it could not be determined if this subject suffered some adverse effect of treatment that led to a reluctance to further cooperate and continue with the study.

Compliance:

No-Data Confidentiality Claims (No claim of confidentiality within the United States), GLP and Quality Assurance Statements were provided. The study was said to be conducted in accordance with all applicable U.S. guidelines as specified in Title 21 of the Code of Federal Regulations parts 50, 556, and 321 and the International Guidelines for Human Testing as promulgated in the Declaration of Helsinki (1964 and as amended 1996).

Institutional Review Board Approval. there were two letters (see pages 134 and 135 of the study report) from the Secretary (or acting secretary) of the MDS Harris Laboratory that indicated that the protocol was approved by the MDS Harris Institutional Review Board. These letters mentioned only minor changes to the protocol. There was no document in the study report that listed the membership or their professional status of the MDS Harris Institutional Review Board.

The study was conducted under the supervision of James C. Kisicki, M.D. who is the Medical Director, Clinical Research Division of the Harris Laboratories in Lincoln, Nebraska.

Dr. Kisicki has Board Certification from the National Board of Medical Examiners and American Board of Family Practice.

Part A. Clinical aspects including AChE Assessments (MRID No.: 44811002).

Experimental Constants

A. Test Materials:

Test Chemical:

Chemical:	DursbanR - technical name for chlorpyrifos
Source:	DOW AgroSciences LLC
Lot:	MM930503-17
Purity:	99.8% (scheduled for decertification in June 2003)
Appearance:	White crystalline powder
CAS #	2921-88-2
Chemical Name:	0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl) phosphorothioate

Negative Control (placebo):

Chemical:	Lactose
Source:	Mallinckrodt N.F.
Lot:	6279 KXHA
Purity:	Not specifically stated but stated to "meets N.F. requirements"
CAS #	63-42-3

B. Human Subjects:

The subjects were recruited from the Lincoln, Nebraska area in response to a advertisement. The criteria for selection for inclusion and continuation in the study were described on pages 102 to 106 of the study report. These principles are briefly described as follows in the Appendix to this DER.

Some of the characteristics of the participants who were actually dosed with either the control (lactose) or chlorpyrifos are as follows:

Number:	30 males and 30 females.
Occupations:	Not stated for each individual.
Age:	Males: 19 to 54 years; Females: 20 to 52 years.
Weight: Males:	146 to 224 pounds; Females: 98 to 200.
Race:	Males: 28 Caucasian, 1 black and 1 Asian; Females: all Caucasian.
Medical History:	Table 4.4 (page 243) presented comments on the medical history of each subject. In the opinion of this reviewer, the medical history appeared to be typical of a group of 60 humans within this age bracket. There did not appear to be any medical conditions that would affect the outcome of the study. The only entries for subject #56 were that she was a non-smoker and was hospitalized twice for childbirth (12/93 and 10/97).

C. Experimental Protocol

The experimental protocol is described in Table 1 below).

Table 1. Experimental Design.

Group	Phrase	Dose (mg/kg)	Subjects	Comments
A	I	0.5	6 ♂/6 ♀	Blood sampled at -10 and 0, hr pretreatment and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120 144 and 168 hours post treatment and analyzed for RBC AChE, chlorpyrifos and its metabolites. Urine, blood pressure, pulse, ECG and body temperature and clinical laboratory assessments were made at pretest and one week after administration of placebo or chlorpyrifos.
B	I	1.0	6 ♂/6 ♀	
D(1)	I	0 (placebo)	6 ♂/6 ♀	
C	II	2.0	6 ♂/6 ♀	
D(2)	II	0 (placebo)	6 ♂/6 ♀	
Total Subjects = 30 ♂ and 30 ♀.				The subjects were released from the study after 7 days.

Phrase I was conducted first and when it was determined that there was no inhibition of RBC AChE and there were no adverse effects, Phrase II was initiated. Phase I groups were dosed on 10/3/98 and Phase II groups were dosed on 10/17/98.

C. Basis for Dose Level Selection:

The low dose of 0.5 mg/kg was based on a previous study (Nolan, 1984) in which this dose level produced decreases in plasma ChE by 80% but had no effects on RBC AChE and did not result in symptoms. This dose was included to provide a NOEL for RBC AChE. The next higher dose of 1 mg/kg was thought to be a dose where noticeable RBC AChE might be expected without showing clinical signs in the subjects. The higher dose of 2 mg/kg was initiated when it was established that there was no inhibition of RBC AChE at 1 mg/kg in order to reach a level where noticeable inhibition of RBC AChE was attained.

D. Dosing Data

Each subject was dose based on his/her body weight and chlorpyrifos was weighed out to the 0.1 mg and placed in a white opaque number zero capsule the day prior to dosing. The capsule was "filled with lactose" and then closed prior to dosing. Data on the actual mg of chlorpyrifos administered to each subject that received the chlorpyrifos were presented in Table 4.27, page 545. This table presents the weight of the capsule, the weight of the capsule plus the chlorpyrifos as well as the weight plus lactose and the subjects weight to assure that each subject was administered the proper dose in mg/kg. For example, subject #56 had a body weight of 87.7 kg and was dosed with 173 mg of chlorpyrifos (268 mg weight of capsule plus chlorpyrifos

- 95 mg weight of capsule). Such that the dose this person in the 2 mg/kg dose group received was $173 \text{ mg}/87.7 \text{ kg} = 1.97 \text{ mg/kg}$.

The subjects arrived at the clinic the day before scheduled dosing and at 8 PM given a "snack" but fasted for the next 10 hours. The subjects were given a bracelet with a unique bar code to identify specimens collected. Starting at approximately 7 AM, each subject was given his/her dose of either lactose only or chlorpyrifos containing capsule. Each subject was watched to assure the capsule was swallowed. Following swallowing, each subject was given 240 mL of water and it was assured by the study team that the capsule was swallowed. The subjects were given their lunch at approximately 11:30 AM and dinner at 4:30 PM and then a snack at 8:30 PM. The subjects were kept at the clinic for 48 hours and were required to return to the clinic at 72, 96, 120, 144 and 168 hours after initial dosing. Although the subjects were asked about strenuous activity prior to coming to the clinic, there were no comments on the level of physical activity the subjects were allowed following dose administration. There were restrictions on the consumption of alcohol and tobacco as well as non-prescription and prescription drugs.

C. Statistics

(Reproduced from the study report page 23-25).

Demographic variables: Descriptive statistics (mean, standard deviation, minimum, maximum, and sample size) were calculated for continuous demographic variables (age, height, and weight) and frequency counts were tabulated for categorical demographic variables (gender, race, and frame size) by gender, by treatment group, and by phase.

Vital signs: For each phase, descriptive statistics were reported for all vital signs measurements (sitting systolic and diastolic blood pressure, pulse, respiration rate, and temperature) at each time point of collection. If a vital signs measurement was re-tested prior to dosing, the re-test value was used, instead of the original value, to calculate the descriptive statistics. However, if a re-test was required for a post-dose assessment, the original value was used to calculate the descriptive statistics. All collected data were included in the summarization of vital signs assessments. Where individual data points were missing, data were summarized based on reduced denominators.

Signs and symptoms: Signs and symptoms were coded using the 5th Edition of the COSTART Adverse Event dictionary. These data were tabulated by the number of subjects reporting at least one symptom and as percent of number of subjects dosed in each treatment group.

RBC AChE: RBC AChE concentrations were presented by subject and blood collection timepoint and were summarized by treatment group and gender using means and standard deviations. RBC AChE percent of baseline concentration values were presented and summarized in the same way. Percent of baseline at time t was calculated as follows:

Percent of Baseline1 = $(\text{AChE} \times 100) / \text{baseline}$

where AChE represents the RBC AChE concentrations at time t and that individual's baseline is the average of the concentrations collected at time -10 and time 0 predose. All collected RBC AChE concentrations were included in the summarization by gender and treatment group. Where individual data points were missing, data were summarized based on reduced denominators. These percent of baseline data were inspected and a depression of 20% or more in any subject's RBC AChE activity, relative to the baseline and in the absence of a concomitant shift in the RBC AChE activity for the control group, was considered a significant depression. The RBC AChE activity was also normalized for both baseline and for RBC AChE activity for the concurrent control (placebo) group for gender at the time interval. Percent of baseline normalized to concurrent control at time t was calculated as follows:

$$\text{Normalized Percent of Baselines} = (100 * \text{AChEt}/\text{baseline}) - \text{control} + 100$$

where AChE and baseline are defined as above and control is the mean percent of baseline response for the control (placebo) group at time t by gender. These data were presented by subject and timepoint and were summarized by treatment group and gender for each timepoint using means and standard deviations.

For the statistical analysis, the normalized data were truncated after 96 hours for Phase 1 and after 48 hours for Phase 2 so that there would be a value for each subject at each time point. Then the truncated normalized data sets from Phase I and Phase 2 were analyzed, separately, using univariate repeated measures analysis of variance (ANOVA) methods and mixed effects modeling to investigate if there were statistical differences attributed to treatment. A univariate repeated measures ANOVA was performed as a split-plot analysis with the gender by treatment combinations, denoted as group, as the whole plot factor and time as the subplot factor. In this setting, the time and group by time interactions are valid only if the data satisfied the sphericity property, which says measurements at all time points are equally correlated. Since this was not expected to be true, statistical tests were adjusted for the violation of the sphericity assumption using Greenhouse-Geisser and Huynh-Feldt adjustments. Mixed effects models were also used where gender and treatment were considered to be fixed factors and time was considered to be a random continuous covariate.

This approach allowed the modeling of the effect of gender and treatment over time in a structured way, providing the opportunity to identify significant higher-order trends associated with AChE inhibition. Covariance structures and terms in the mixed effects models were tested using likelihood ratio tests comparing nested models. Covariance structures failing to provide adequate fit and terms not statistically significant at the 0.10 level of significance were excluded from the model. Differences among the treatment by gender combination pairs were tested using contrast statements within the final mixed effects model and were considered statistically significant at the 0.05 level of significance.

All summarization and analyses were conducted using SAS. Output from all SAS programs are included in Appendix 3, Supporting Statistical Documents.

Specific Methods and Results

1. Comments on the Subjects and Their Participation During the Course of the Study.

There were sixty individuals (30 males and 30 females) enrolled in the study that were actually dosed with chlorpyrifos (36 subjects, 18 males and 18 females) or placebo (24 subjects, 12 males and 12 females). Of the 60 original subjects selected, 14 were replaced by alternates prior to administration of the dose for various reasons. This is not considered to affect the outcome.

Two subjects, one female placebo (#32) and one female dosed with 2 mg/kg (#56) did not complete the study. The former did not return for the day 6, 7 or 8 post dosing assessments and was discontinued as per the SOP. Since this subject was in the placebo group, the dismissal of this subject is less consequential. The female (#56) dosed with 2 mg/kg chlorpyrifos was described as having a personal conflict and did not return on days 4, 5, 6, 7 or 8 for post dosing events. This subject will be discussed in the later sections of this review in more detail.

A third subject (#11), a male dosed with 1 mg/kg did not provide a blood sample at 168 hours since he was out of the area at the time. He returned to the clinic for subsequent follow up investigations (physicals) and was not dismissed from the study. This is not considered consequential because 168 hours is well past the expected time of effect of chlorpyrifos.

One subject (#6), a male in the placebo group consumed alcohol on post treatment days 6 (equivalent to one Margarita). This is not considered consequential to the interpretation of the study.

Five subjects (#s 35, 43, 49, 50 and 60) either failed to provide urine samples for a given interval or did not collect their samples properly such as failure to collect or did not collect in the proper container. This is not considered consequential to the assessment of AChE but may be relevant to the pharmacokinetic studies.

2. Clinical Signs and Electrocardiogram

The signs and symptoms were coded according to the 5th Edition of the COSART Adverse Event Dictionary.

The subjects were instructed to inform the physicians and/or nurses of any signs or symptoms that they experienced. They were also asked if they felt that they received the test material and if they did why. Vital signs included blood pressure, pulse, respiration and body temperature and were assessed on the morning prior to dosing and at 1, 2, 4, 8, 12, 24, 48 and 168 hours post dosing. The observers that examined the subjects classified symptoms as

possibly and/or probably related to treatment if a symptom was noted within 48 hours of treatment. Since the observers did not know if a subject actually received chlorpyrifos, many of the subjects receiving the placebo were said to have symptoms possibly or probably related to the test material.

Appendix Table 1 shows the signs that were indicated by the subjects in either the placebo or groups dosed with 0.5, 1 or 2 mg/kg of chlorpyrifos.

Electrocardiograms (ECG) were done on screening and one week after administration of the placebo or chlorpyrifos. Table 4.10 (pages 345 to 351) presented the data for these analyses. All result entries were reported as "normal". There were some subjects with "sinus bradycardia" noted at week one post dosing that was not present in the screen but one of this occurred in the placebos as often as in the treated subjects. There were no indications of abnormal recordings to indicate that chlorpyrifos affected the heart. ***The one week interval between the time of test material administration and ECG assessment is considered by this reviewer too long to be meaningful for a response to an OP. A more appropriate time would be the expected time for peak RBC AChE inhibition.***

The study author asserted that "There were no differences noted during this study attributed to treatment with regard to signs or symptoms, vital signs or clinical laboratory results". HED reviewers concur with this conclusion. There is no indication that a symptom in the treated groups was any greater than similar symptoms in the placebo groups. Other signs occurred at times that did not coincide with any indication of inhibition of RBC AChE.

3. Clinical Chemistry, Hematology and Urinalysis.

Clinical chemistry, hematology and urinalysis assessments were made at pretest screening and one week after dose administration. ***The one week interval following dosing and clinical chemistry, hematology and urinalysis is not considered by this reviewer to be the most appropriate time. A more appropriate time would be closer to the time for expected peak effect of AChE inhibition.***

Serum chemistry included: ALT, AST, albumin, alkaline phosphatase, calcium, chloride, cholesterol, creatinine, GGT, glucose, inorganic phosphorous, LDH, potassium, sodium, total bilirubin, total protein, urea nitrogen and uric acid.

Hematology included: basophils, eosinophils, HCT, Hgb, lymphocytes, monocytes, neutrophils, platelet count, RBC and WBC

Urinalysis included: bacteria, bilirubin, blood, casts, glucose, ketones, leukocyte esterase, nitrite, protein, RBC, specific gravity, urobilinogen, WBC and pH.

Inspection of the data tables (pages 413 to 454) did not indicate that these parameters were affected by chlorpyrifos treatment. It should be noted, however, that subject #56, the only

subject showing inhibition of AChE did not provide blood or urine samples for clinical chemistry, hematology or urinalysis.

Note: Additional tables presented the results of urinary analysis for drugs (cocaine, ethanol and cannabinoids) taken from samples at screening and "interim". All entries were indicated as being negative. Data were also presented for the results of the pregnancy test and all entries were negative.

4. AChE Assessments.

Analytical Methods for AChE assessment.

Blood was withdrawn via venipuncture on an arm vein and there were a total of 14 blood specimens collected per subject (if they completed the study). Once drawn, the blood samples were centrifuged for 12 minutes at 3000 rpm and the plasma discarded (except for the prescreening sampling). The erythrocytes were then washed with saline and centrifuged for an additional 12 minutes at 3000 rpm. The saline was discarded and the erythrocytes were washed twice by the same procedure. The resulting erythrocytes were stored frozen and shipped on dry ice from the clinical facility located in Lincoln, Nebraska to the MDS Clinical Laboratory located in Toronto, Ontario Canada. AChE was assessed by the Ellman method using acetylthiocholine as the substrate. The analysis was made using a Hitachi 917 automated analyzer. Appendix 5 (pages 547 to 573) described in detail the methods for the preparation and assay and mean data for assessment of the RBC AChE by the Laboratory. It was noted that the standard protocol for this assay called for initially centrifuging the whole blood for 30 minutes (see page 552) and not the 12 minutes as indicated for the subject's samples in this study. This difference is noted but it is not considered to impact the interpretation of the data.

An overview of the results of the RBC AChE assessments is presented in Table 2. Comments on Table 2 are as follows.

Precision of the data.

The mean data for RBC AChE in U/L for the untreated subjects when assessed at 0 hour ranged from 8143 ± 982 ($\pm 12\%$) to 9572 ± 539 ($\pm 6\%$) for the five different groups. For females the range was 8576 ± 556 ($\pm 6\%$) to 9165 ± 709 ($\pm 8\%$). The highest mean for both males and females was found in Group B scheduled for dosing with 1.0 mg/kg with chlorpyrifos.

The mean values for males and females was from about 8000 to 9500 U/L are within the expected range for human RBC AChE. In general, the analytical aspects of the measurement of RBC AChE are considered appropriate.

Indications of RBC AChE Inhibition Based on Comparison of the Means.

I. **Males.** Column three of Table 2 shows that one of the two male *placebo* groups had means as low as 10.2% less than the predosing mean and this difference was noted at 144 hours

post dosing. *The treated males* based on mean data had maximum decreases of -10.7%, -13.7% and no differences for the 0.5, 1.0 and 2.0 mg/kg dose groups respectively when compared to the predosing values. These maximum depressions of the means were at 96 hours for both the 0.5 and 1.0 mg/kg dose groups. Since chlorpyrifos is an organophosphate, it would be expected that inhibition would start to occur and remain from about 2 hours to over 24 hours. Thus, 96 hours is well past the critical hours for expected start of inhibition.

ii. Females. Among females, the two control groups demonstrated that there was as much as a -9.6% decrease in RBC AChE and maximum difference was again at 96 hours. The treated groups demonstrated -6.8%, -8.7% and -10% (for all subjects but only -6.2% when one subject that did not complete the study is not included) for the 0.5, 1 and 2 mg/kg dose groups. Thus, the decreases in the means for all treated groups are near the maximum decrease that was noted in the placebo group.

In conclusion, when means are considered and compared to the predosing values, there is no support for a conclusion that chlorpyrifos inhibited the RBC AChE in either males or females. This is especially true when one subject that was demonstrated to have inhibition is not included in the comparison (see below).

Table 2. Overview of the results of the RBC AChE assessments.

Group	Mean Predosing (Hour 0) U/L	Lowest Posttreatment Mean (U/L)	Highest Posttreatment Mean (U/L)	Lowest % for an individual
D: Control 1 Males	8143±982 (12%) ¹	7316±598 -10.2% @ 144 hr ²	8043±705 +2% @ 12 hr	88.36% @ 144 hr (#10) ³
Females	8619±861 (10%)	7795±759 -9.6% @ 96hr	8817±884 +2.3% @ 8 hr	83.26% @ 6 hr (#34)
A: 0.5 m/kg Males	8998±730 (8%)	8036±738 -10.7% @ 96 hr	9189±924 +2.1% @ 8 hr	82.68% @ 96 hr (#9)
Females	8612±1160 (13%)	8028±917 -6.8% @ 144	8938±917 +3.8% @ 144 h	88.62% @ 36 hr (#19)
B: 1.0 mg/kg Males	9572±539 (6%)	8263±399 -13.7% @ 96 hr	9862±865 +2.9% @ 4 hr	75.47% @ 96 hr (#11)
Females	9165±709 (8%)	8366±499 -8.7% @ 168 hr	9396±517 +2.5% @ 4 hr	88.99% @ 144 hr (#33)
D: Control 2 Males	9171±1112 (12%)	8999±1020 -2% @ 36 hr	9425±1041 +2.8% @ 72 hr	89.71% @ 144 hr (#44)
Females	8576±556 (6%)	8380±422 -2.2% @ 4 hr	8941±442 +4.3% @ 72 hr	88.40% @ 144 hr (#50)
C: 2.0 mg/kg Males	8608±896 (10%)	8584±806 (no difference)	9004±840 +4.6% @ 72 hr	89.97% @ 36 hr (#48) 71.77% @ 12 hr
Females	8623±855 (10%)	7761±1239 -10% @ 12 hr. 8089±1088 -6.2% @ 12 hr ⁴	8604±1064 (no difference)	(#56) 92.82% @ 12 hr (#58) ⁴

Data are from 5.1.1 and 5.1.2 for Groups A, B, and Control 1 and 9.1.1 and 9.1.2 for Group C and Control 2.

¹ The number in () is the standard deviation as percent of the mean. ² The percent is the difference

between the highest or lowest mean vs. the control at the interval given.³ The individual subject number for the person showing the lowest relative reading for AChE and the interval that this reading was made.

⁴ Since subject 56 did not complete the study, the reading for the second lowest subject is also presented for this group or the mean for the remaining 5 subjects is presented.

Indications of RBC AChE Inhibition Based on individual Responses.

Comment. There is an apparent decrease in the activity noted in Phase I for the males and/or females in the placebo, 0.5 and 1 mg/kg dose groups since at hour 96 many of these (placebos included) suddenly have readings lower than their previous readings for RBC AChE. There was no explanation for this provided. See Table 5.1.2 on page 60 of the study report. Since hour 96 is well past the time when chlorpyrifos would be expected to cause inhibition, the impact of this sudden decrease in activity is not such as to seriously impact the interpretation of the data. This sudden decrease in activity was not noted for Phase II of the study when both placebo's and 2 mg/kg chlorpyrifos were tested (see Table 9.1.2 page 79 of the study report).

Males. Column five of Table 2 shows that for the control group males, at least one individual demonstrated as much as 11.6% (subject # 10) or 10.3% (subject # 44) less than his predosing value. Among the treated groups there was a maximum of 17.4% (subject #9), 24.5% (subject #11) and 10% (subject # 48) apparent decrease in RBC AChE for the 0.5, 1.0 and 2.0 mg/kg dose groups, respectively. Since there are subjects in both the 0.5 and 1.0 mg/kg dose groups that have apparent decreases in AChE it can be implied that these individuals may be unusually sensitive to inhibition of AChE by chlorpyrifos. Thus, the following will discuss in more detail the overall response of these individuals throughout the study. Selected other subjects will also be discussed for the reasons given below.

Subject # 9. Group A. (0.5 mg/kg chlorpyrifos treatment). Subject # 9 was a 19 year old Caucasian male 75 inches tall and weighing 210 pounds and was a non-smoker. No clinical signs or abnormalities were noted in this person's clinical chemistry or ECG assessments. This subject was reported as having intestinal cramps starting at 2 days, 22 hours and 44 minutes post dosing that lasted 15 hours. This subject replied "no" when asked if he had received the test material and not the placebo.

This subject had RBC AChE readings (in U/L) of 8294, 8602, 8437, 8646, 8844, 8723, 8580, 8261, 8107, 8250, 6985 (-19%), **7535 (-12%), 7376 (-14%) and 7700 (-11%)** for the -10, zero, 2, 4, 8, 12, 24, 36, 48, 72, **96, 120, 144 and 168** hour assessment times respectively. Thus, indicating that the apparent decrease in RBC AChE did not begin until 96 hours after dosing. It is noted that the cramps did not coincide with the lower AChE levels. It was noted above that there is a problem with the assays after hour 96 (see comment above).

At the onset of this subject's intestinal cramps (~72 hours) his RBC AChE was at most 3% less than the zero time reading. However, at 96 hours, his RBC AChE was lowest but his cramps had already disappeared by 86 hours. Refer to page 467 of the study report for information on the onset and duration of the cramps.

In conclusion, there is no justification to support that this individual was unusually sensitive to inhibition of RBC AChE. Inhibition would be expected to appear within a few hours (~ 4-8 hours) post dosing and not 96 hours later. There were also no symptomatic expressions indicative of AChE inhibition (i.e. intestinal cramps are not a specific response to a AChE inhibitor and could be due to many things).

Subject #11. Group B (1.0 mg/kg chlorpyrifos treatment). Subject # 11 was a 45 year old black male 71 inches tall and weighing 191 pounds and smoked one and a half packages of cigarettes a day. This subject did not report for his day 7 examinations or blood sampling. He did not submit urine for the 144 to 156 hour or 156 to 168 hour collection intervals. He returned to the clinic "the following week" for a post physical exam, ECG and laboratory work up. There was no record of how he responded to whether or not he thought he had received the test material rather than the placebo. This subject's clinical laboratory work up for post treatment indicated he was "moderately lipemic".

On two occasions (refer to page 468 and Appendix 4.21/1) this subject was reported to have a headache. The first occasion began 3 hours and 40 minutes post dosing and lasted for 19 hours. This means the headache started at 11 am on October 3 and lasted until 6 am on October 4. The second occasion began 22 hours and 40 minutes (approximately 6 am on October 4 post dosing and lasted 1 day and 9 hours. [There seems to be a reporting glitch here because the first headache is reported to be resolved at 6:00 and the second headache starts at 6:00 both on October 4, 1998. There may actually be just one headache.]

This subject had RBC AChE readings (in U/L) of 10307, 10098, 9097 (-10%), 10318, 9768 (-3%), 9779 (-3%), 9977, 9768, 9141 (-10%), 10153, 7700 (-24%), 9185 (-9%), 9218 (-9%) and not assessed for the -10, zero, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120 144 and 168 hour assessment times. Thus, there was considerable variation in this individual's readings for RBC AChE but they do not fit an expected pattern of inhibition by an organophosphate. Since maximum apparent decrease occurred at 96 hours post dosing (again there is a problem with the hour 96 readings) and the reading at 72 hours was 10153 or essentially the same as the predosing zero hour, this subject is not considered to have chlorpyrifos related inhibition of ChE.

It is noted that there was no consistent depression of RBC AChE during this interval when the subject experienced his headache. For example, the readings were -10%, similar, -3%, -3%, -3%, and -3% at 2, 4, 8, 12, 24 and 36 hours respectively.

In conclusion, there is no justification to support that this individual was unusually sensitive to RBC AChE inhibition by chlorpyrifos. The headache that this individual was reported as having did not coincide with decreased levels of RBC AChE and it was not present when AChE was lowest.

Subject #47. Group C (2 mg/kg chlorpyrifos treatment). Subject #47 was a 24 year old Caucasian male 69 inches tall and 184 pounds. He responded "no" to the question about whether or not he thought he received the test material and did not have any obvious symptoms of AChE

intoxication. This subject had highest "estimated dose received" (1.130 mg/kg compared with the group mean of 0.59 mg/kg, see Table 16, page 47 of the Pharmacokinetics report) based primarily on plasma and urinary content of TCP. This subject did not have indications of AChE inhibition since there was at best a 5% decrease in activity at 24 hours but the preceding and succeeding assessments were both higher than the baseline.

Subject #48. Group C (2.0 mg/kg chlorpyrifos treatment). Subject # 48 was a 23 year old Caucasian male 71 inches tall and weighing 162 pounds and smoked 10-14 cigarettes a day. He responded no to the question about whether or not he thought he received the test material. According to the data table on page 471, this subject did not experience any symptoms during the post dosing phrase.

This subject had RBC AChE readings of 8569, 8206, 8019, 8008, 7887, 7689, 8206, **7546** (-8%), 7832, 8074, 7766, 7766, 7942 and 8184 for the -10, zero, 2, 4, 8, 12, 24, 36, 48, 72, 96, 144 and 168 assessment times respectively. Thus, this subject's maximum decrease at 36 hours was immediately proceeded by a value actually the same as the zero time assessment and thus does not show a pattern consistent with inhibition by an organophosphate.

Females. Column five of Table 2 shows that for the control group females, at least one individual demonstrated as much as 16.7% (subject # 34) or 11.6% (subject # 50) less than her predosing value. Among the treated groups there was a maximum of 11.4% (subject #19), 11% (subject #33) and 28.2% (subject # 56) apparent decrease in RBC AChE for the 0.5, 1.0 and 2.0 mg/kg dose groups. Since the subjects in both the 0.5 and 1.0 mg/kg dose groups did not exceed the placebo group for apparent decreases in AChE it is not implied that there are individuals in these dose groups that may be unusually sensitive to inhibition of AChE by chlorpyrifos. Subjects # 51 and 56 are discussed as follows.

Subject #51. Phase II Group C female, 2 mg/kg chlorpyrifos. Refer to Table 4 below for AChE data on this subject. This subject *sustained* a decrease of 5 to 6% RBC AChE over the critical hours of 12, 24, and 36. This subject also had one of the highest levels of TCP in her blood. But the peak level of TCP did not correspond with the lower readings for AChE.

The placebo female group for Phase II was demonstrated to be very stable over the course of the seven days on the study (see last column in Table 4) as indicated by the data from one individual which demonstrated the most variation of the six females in the placebo group. Overall, the 5-6% lower reading is noted but not considered to be definite inhibition caused by chlorpyrifos.

5. **Special considerations for subject #56 who did not complete the study.**

Subject # 56. Group C, 2 mg/kg chlorpyrifos. As indicated previously, subject # 56 dropped out of the study after 48 hours. It was reported that repeated attempts to contact this person were unsuccessful and no post study medical evaluation was performed. Page 472 of the study report indicates that this subject reported experiencing "numbness of the upper arms" starting 2 hours and 24 minutes after administration of the chlorpyrifos and lasting a total of 42

minutes. On page 510, the table listing the subject's response to the question if they thought they received the test material does not include subject #56.

This subject was the only subject on the study that demonstrated apparent RBC AChE inhibition. Table 3 below provides details about this subject and shows the extent of inhibition at the critical time interval. It is apparent that this individual's RBC AChE is decreased starting at 8 hours and remaining so until 48 hours when she was last tested.

Table 3. Summary of Subject #56

Parameter	Results	Comments
Description (a)	A 34 year old Caucasian female 72 inches tall and weighing 193 pounds and was a non-smoker. This individual did not report for the day 4, 5, 6, 7 or 8 blood collection or assessment of vital signs and did not provide urine samples after 48 hours.	There is nothing obviously different about this subject to suggest an unusual susceptibility to chlorpyrifos. The subject was genotype QQ but about half the persons on the study had this genotype.
Symptoms (b)	Subject had a transient "numbness in upper arms" that started to occur 42 minutes after dosing and lasted for 2 hours and 24 min (self resolved without medication).	The numbness alone does not seem to be a response to a AChE inhibitor and since this occurred and was resolved by three hours or before there was appreciable apparent inhibition of AChE at 8 hours post dosing.
RBC AChE IU/L (c)	8426 at -10 hours 8910 at 0 hour 8668 predosing mean 8602 (no difference) at 2 hours, 8525 (-2%) at 4 hours, 6688 (-23%) at 8 hours, 6221 (-28%) at 12 hours 6424 (-26%) at 24 hours 7051 (-19%) at 36hours 6882 (-21%) at 48 hours No additional assessments.	Subject shows decrease in RBC AChE that can be considered the critical times expected for an OP following a oral administration. No other subject shows a similar pattern of decrease in activity.
Serum chlorpyrifos (d)	18 ng/gm at 8 hours 2.5 ng/gm at 12 hours All other times - not detected (< 1 ng/gm) The highest level in all other subjects was only 5.6 in a female (#21) dosed with 1 mg/kg.	The serum chlorpyrifos data and the serum and urine TCP data all indicate that subject # 56 absorbed chlorpyrifos to a greater extent than the other subjects in this study.
Serum TCP (0 to 48 hours)	Subject has total of 6671 ng/gm TCP in serum with values of 71, 120, 1600, 1500, 1300, 1100	

Parameter	Results	Comments
(e)	and 980 ng/gm for the 2, 4, 8, 12, 24, 36 and 48 hour intervals. Note there was little TCP in blood at 2 to 4 hours or the time the numbness was noted. This is the highest for the group dosed with 2 mg/kg since the range for all others (both sexes) was 1404 (#42) to 5860 (#47).	
Urine TCP (f, corrected for creatinine) (0 to 48 hours)	Subject had a total of 22.8 mg or was highest for the group dosed with 2 mg/kg since the range for the others in this group was 4.8 (#40) to 16.2 (#47) with a mean of 8.4.	
Estimated dose of chlorpyrifos (g)	0.640 mg/kg. - from Table 16 Compares favorably with the group mean of 0.59 ± 0.21 mg/kg (range 0.323 (#40) to 1.130 (#47) mg/kg.	Revise - needs back calculation.
CPOase (h)	Subject has reading of 9278 Units/liter comparing favorably with the group mean of 9523 ± 1517 Units/liter.	There is nothing unusual about the serum activity of CPOase, paraoxonase or diazoxonase and subject was described as phenotype "QQ low" which was a typical classification for many subjects in the study.

(a) Data are from Appendix 4.3, page 242.. (b) Data are from Appendix 4.21.1, page 472. (c) Data are from Table 4.13.2, page 407. (d) Data are from Table 5, page 34 of the Pharmacokinetics report. (e) Data are from Table 7, page 36 of the Pharmacokinetics report. (f) Data are from Table 14b (corrected for creatinine), page 45 of the Pharmacokinetics report. (g) Data are from Table 16, page 47 of the study report. (h) Data are from Table 17, page 50 of the Pharmacokinetics report.

It is noted that in the screening study indicated that this individual had a RBC AChE of 7678 U/L. This was listed as a clinical laboratory comment as "not significant in the context of this study". When compared to the mean for the -10 and zero hour samplings (mean = 8668 U/L), this would be 11% less. This value of 11% less it is not as much as the approximately 28% decrease that was noted for this person following treatment with 2 mg/kg chlorpyrifos. Thus, the decreases in AChE after 8 hours are greater than the spontaneous variation for this subject.

This subject has inhibition rather than random decrease in activity since the decrease in activity starts at 8 hours and rises to a peak at 12 hours and then appears to start to decline at 24,

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36 and 48 hours. This would be expected for an organophosphate AChE inhibitor. The pattern of decreased activity also correlates with the amount of TCP in the serum as shown in Table 4.

Table 4. Correlation between RBC AChE activity and blood level of TCP in humans dosed with 2 mg/kg chlorpyrifos.

Hour	Subject #56_ RBC AChE	TCP Blood	Subject #47_ RBC AChE	TCP Blood	Subject #51_ RBC AChE	TCP Blood	Subject #48_ RBC AChE	TCP Blood	Placebo _ #44 RBC AChE	Placebo _ #60 RBC AChE
2	=	71	+2%	580	-2%	420	-4%	180	-1%	-3%
4	-2%	120	+2%	640	-1%	580	-5%	250	=	-5%
8	-23%	1600	=	790	-2%	1300	-6%	240	-3%	+1%
12	-28%	1500	=	910	-6%	690	-8%	260	-6%	+2%
24	-26%	1300	-5%	980	-5%	540	-2%	380	-6%	-1%
36	-19%	1100	=	960	-5%	750	-10%	460	-4%	=
48	-21%	980	-1%	1000	-3%	730	-7%	470	-5%	=
72	Subject did not return for additional sampling.		+4%	690	+1%	600	-4%	300	+1%	-3%
96			+2%	460	+4%	420	-7%	210	-4%	-3%
120			+7%	320	+6%	150	-7%	140	-4%	=
144			+2%	220	-2%	170	-6%	77	-10%	-3%
168			+7%	150	=	110	-2%	48	=	+1%
Net TCP	0 to 48 hrs 6671 0 to 168 hrs - no data		0 to 48 hrs 5860 0 to 168 hrs 7700		0 to 48 hrs 5010 0 to 168 hrs 6410		0 to 48 hrs 2240 0 to 168 hrs 3015		N/A	N/A
	Definitely shows inhibition correlating with blood level of TCP.		Highest levels of TCP do not correlate with ↓RBC AChE.		Sustains a decrease at critical times and is thus <i>possibly</i> showing inhibition.		Subject has lowest level of blood TCP but seems to sustain a decrease in RBC AChE		Shows that varies to -10%.	Shows that female RBC AChE is stable.

TCP data are from Table 7 page 36 in MRID No.: 45144101 and are ng/gm. Note: TCP data were not reported for the blood from the placebo group in Table 7 of the study report. RBC AChE data are from Table 9.1.2 page 78 from MRID No.: 44811002 and are the percent decrease (-) or increase (+) in RBC AChE relative to the baseline. The = sign indicates that the assessment was within ±1% of the baseline.

Table 4 also presents data on the variation of RBC AChE activity in one other female and two males dosed with 2 mg/kg of chlorpyrifos and the amount of TCP in their blood. In addition, a male and a female from Phase II placebo group selected because they showed the

most decrease in activity at any post dosing assessment time are also included to show the spontaneous variation of RBC AChE. It is noted that the females are stable but the male showed decreases. One other male (Subject #41) also showed what appeared to be sustained decreased activity.

Table 4 indicates that female subject #51 sustains a slight decrease in RBC AChE and also has a high blood level of TCP but not at the critical times of lower AChE readings. Therefore this subject is not considered to show definite evidence of RBC AChE inhibition in response to 2 mg/kg of chlorpyrifos since the level of decrease is too low and not coincident with serum TCP. .

Part B. Pharmacokinetic and Paraoxonase Data (MRID No.: 45144101).

Part B 1. Pharmacokinetic data.

The sixty subjects on this study gave blood samples (5 mL) for the purpose of determination of chlorpyrifos, chlorpyrifos oxon and 3,5,6-trichloro-2-pyridinyl (TCP) at -10 hours prior to dosing, at zero hour prior to dosing and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hours post dosing. Urine samples from all urine voided (as per the protocol) for the intervals -48 to -36 hours, -36 to -24 hours, -24 to -12 hours, -12 to 0 hours pretreatment and 0 to 6 hours, 6 to 12 hours, 12 to 24 hours, 24 to 36 hours, 36 to 48 hours, 48 to 60 hours, 60 to 72 hours, 72 to 84 hours, 84 to 96 hours, 96 to 108 hours, 108 to 120 hours, 120 to 132 hours, 132 to 144 hours, 144 to 156 hours and 156 to 168 hours post treatment. The blood and urine were analyzed by GC/MS for chlorpyrifos, its oxon analog and TCP and the method of preparation using internal standards was described. TCP was derivitized prior to analysis.

Data were presented that demonstrated that the recovery of chlorpyrifos 98.0 to 99.8%), chlorpyrifos oxon (95.4 to 98.0%) and TCP (91.1 to 97.1%) and that the lower limit of detection was about 1 ng/gm of blood for chlorpyrifos and its oxon analog but was about 12.1. ng/gm for TCP. Similarly, recoveries of from 96 to 104% for these chemicals from urine were demonstrated. Additional data demonstrated the stability of these chemicals in blood and urine stored -20 or -80 °C for 6 or 10 days were provided.

Blood and urine analysis for chlorpyrifos and chlorpyrifos oxon.

Chlorpyrifos and chlorpyrifos oxon were *not* found in any of the urine samples from any of the subjects (refer to Table 10 of the study report page 39). Chlorpyrifos oxon was not found in the blood of any subject (refer to Table 6 of the study report page 35).

Chlorpyrifos was found only occasionally in the blood (refer to Table 5 page 34 of the study report). No subjects dosed with 0.5 mg/kg chlorpyrifos were found to have chlorpyrifos in their blood. Subjects 11 (1.0 ng/gm at 2 hours), 14 (2.7 and 1.5 ng/gm at 4 and 8 hours), 21 (5.6 and 2.9 ng/gm at 2 and 4 hours) and 30 (1.0 ng/gm at 8 hours) dosed with 1 mg/kg were

noted to have chlorpyrifos in their blood at the assessment times as indicated. Subjects 47 (3.1, 1.3, 3.4 and 1.8 ng/gm at 2, 4, 8 and 12 hours), 49 (3.1 and 1.7 ng/gm at 2 and 8 hours), 56 (15.0 and 2.5 ng/gm at 8 and 12 hours) and 59 (2.2, 4.1, 4.1 and 1.5 ng/gm at 2, 4, 8 and 12 hours) dosed with 2 mg/kg were also shown to have chlorpyrifos in their blood. These are very small amounts of chlorpyrifos. The highest level was found in subject #56 at 8 hours.

Blood and urine analysis for TCP.

TCP was detected in the blood of *all* subjects dosed with chlorpyrifos (refer to Table 7 page 39 of the study report). Table 4 summarizes the mean data generated for TCP analysis and includes selected data for subject #56.

Table 4. TCP content of blood and urine and estimated half life and dose received. .

Dose	Blood Data AUC(0-∞)	Half Life	Urine Data AUC (0-∞)	Half Life	Estimated Absorbed (mg/kg)
0.5 ♂	12.8±4.2	29.7±7.4	4.2±0.7	29.8±8.9	0.17±0.10 (~35%)
♀	20.1±9.1	44.5±32.4	5.8±3.5	38.8±24.3	
1.0 ♂	27.2±11.5	27.7±5.6	10.9±4.7	28.5±6.9	0.31±0.10 (~31%)
♀	25.3±10.2	30.5±7.4	7.5±2.9	33.6±7.4	
2.0(b) ♂	60.7±24.7	36.2±6.9	18.9±6.8	38.1±6.5	0.59±0.21 (~30%)
♀	49.0±19.7	35.2±4.8	14.9±3.6	32.8±6.5	
#56♀	53.6 0 to t	Not calculated	20.8 0 to 48 hr	Not Calculated	0.64 mg/kg or 1.76 mg/kg (b)

Blood data are from Table 9 page 38 and urine data are from Table 15 page 46.

(a) Estimated dose absorbed based on the group mean in mg/kg for both sexes combined.

Data are from Table 16 page 47.

(b) Obtained by calculation on page 27 of the study report. This would suggest that this person was not overdosed.

(b) mean data for the 2 mg/kg dose group females does not include subject #56.

The number in () is the percent of the nominal dose which was supposedly absorbed.

AUC = area under the curve.

The plasma and urine data show that chlorpyrifos is absorbed to the extent of about 30 to 34% as indicated by the recovery of TCP the principle metabolite. There were problems with subject #56 since and the half life in neither blood or urine could be calculated in a comparable

fashion since this subject left the study after 48 hours.

The extent of absorption is less than the earlier report from Nolan (1984) and this may be attributed to the fact that the chlorpyrifos in this study was administered in a capsule and this would lead to slower absorption.

Additional serum TCP data on selected subjects is presented in Table 4.

Part B. 2. Serum Paraoxonase and chlorpyrifos oxonase (CPOase) Assessment.

A 5 mL blood sample was taken prior to dosing and sent to the laboratory of Dr. Clayton Furlong, Department of Medicine and Genetics, University of Washington, Seattle, Washington and assessed for paraoxonase activity using a spectrophotometric assay. Paraoxonase was tested with chlorpyrifos oxon, paraoxon, or diazoxonase. When the test substance was chlorpyrifos oxon, the activity was called CPOase.

Table 17 of the study report presented data for each subject and group means for the subjects dosed in both phase I (October 3, 1998) and II (October 17, 1998). Table 5 summarizes the CPOase data in this study. Paraoxonase (range - ~250 to 2502 units, when paraoxon was the substrate) and diazoxonase (range ~6548 to 22452 units, when diazinon oxon was the substrate) data were also presented but not shown in this review since the subject of the review is chlorpyrifos.

Group	CPOase	Range	Comment
Phase 1 Control	8418±1639	5430 to 10410	5 subjects were QQ (low), 5 were QR (medium). And 2 were "QQAow". Both the high and low readings were from OR individuals.
Phase 1 0.5 mg/kg	7965±1536	5412 to 13341	% were QQ and 7 were QR. The low was from a QQ and the high was from a QR.
Phase 1 1 mg/kg	8463±1386	6599 to 11615	6 were QQ, 5 were QR and 1 was RR. The high value was from a QQ and the low value was also from a QQ.
Phase 2 Control	11357±3097	6904 to 17333	6 were QQ and 5 were QR and one was RR. The high value was from the RR person and the low value was from a QQ person.
Phase 2 2 mg/kg	9523±1517	7048 to 13018	6 were QQ, 5 were QR and 1 was RR. The high value was from a QR person

			and the low value was also from a QR person. Subject # 56 has a value of 9278 Units/liter and is very close to the group mean and thus does not have an unusual ability to metabolize chlorpyrifos oxon.
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Conclusions, Discussion, Study Deficiencies and Classification.

1. Study author's conclusions.

The study author (page 10 of the study report) concluded "The NOEL for signs and symptoms in fasted human following a single oral dose was 2.0 mg chlorpyrifos/kg of body weight. The NOEL for RBC AChE inhibition in humans given a single oral dose of chlorpyrifos was 1.0 mg/kg of body weight. The 2.0 mg chlorpyrifos/kg of body weight dose level represented a threshold in that it produced greater than 17.3% depression in 1 of 12 volunteers."

2. Reviewer's conclusion and discussion.

Signs and Symptoms. There was no indication that any of the signs and symptoms that were noted in the subjects dosed with chlorpyrifos were different in either their nature or degree of severity from the signs and symptoms that were noted in the placebo groups. Some reported symptoms in the subjects that were dosed with chlorpyrifos occurred either well before or after the time when chlorpyrifos absorption was maximum or when the blood or urine level of TCP the principle metabolite of chlorpyrifos was at its maximum level. Also, the only subject showing definite inhibition of RBC AChE did not display any symptoms when maximum inhibition of AChE was attained and this subject's only reported sign occurred and resolved well before her RBC AChE was shown to be inhibited and before her serum level of TCP reached higher levels. If the "upper arm numbness" was related to some other cause due to chlorpyrifos than inhibition of RBC AChE it would still be expected for the numbness to persist while the absorption of chlorpyrifos increased. Thus, the NOAEL for symptoms is > 2 mg/kg chlorpyrifos.

Pharmacokinetic Data. Based on the pattern of occurrence of TCP the principle metabolite of chlorpyrifos in the blood and urine, chlorpyrifos is absorbed rather slowly from the gut and requiring about 8 to 12 hours for the TCP blood level to reach a maximum. Based on the blood level of TCP, subject # 56 absorbed much more chlorpyrifos in the first 48 hours and this subject also had clear evidence of RBC AChE inhibition.

The rate and extent of absorption of chlorpyrifos in this study was slower and lower than reported in an earlier study (Nolan, 1984) and this was attributed by the study author to be due to

the differences in administration. In this study, chlorpyrifos was administered in a capsule but in the earlier study it was administered on top of a lactose pill. The need for the capsule itself to dissolve in the g-I tract may be a factor. The slower rate of absorption in this study coupled with the high rate of metabolism of chlorpyrifos as indicated by failure to find either chlorpyrifos (except for occasional findings) or its oxon in the blood could result in differences in the extent of inhibition that would result from a technique for administration that provides for faster absorption. This possibility should be taken into account when the 1984 study is compared with this present study.

RBC AChE Inhibition. Review of this study identified one subject (#56, a female dosed with 2 mg/kg) as demonstrating inhibition of RBC AChE. The decreases in RBC AChE were shown to correlate with high levels of TCP in her serum. As much as 28% inhibition was noted at 12 hours post dosing.

A second subject (#51) was considered as possibly also showing inhibition and also had one of the highest levels of serum TCP for the group dosed with 2 mg/kg. Although this subject had decreases to only 5 to 6% of her predosing mean, this level of decrease was sustained over the critical hours of expected time for an OP to inhibit blood AChE (i.e. 8 to 24 hours). Inspection of the other four females in Phase II indicated that their RBC AChE activity was very stable over the 168 hour post dosing period. Overall, subject #51 is considered to have only very minimum and indefinite inhibition.

None of the males were determined to be showing definite decreases in RBC AChE. It was noted that one subject (# 48) had decrease of 8% at 12 hours and 10% at 36 hours but two placebo males indicated that variation in RBC AChE activity can also vary to a decrease of 10%. Also subject # 48 had one of the lowest net totals of TCP in his blood for the group receiving 2 mg/kg.

Study Classification.

This study is being classified as RESERVED since there are no current guidelines for classifying studies with human subjects.

4. Study deficiencies and compromising issues.

-Plasma ChE was not assessed for. This is considered important since other recent studies with humans and OPs include plasma ChE assessment. Also it is known from the earlier study with humans that plasma ChE is inhibited at lower doses than RBC AChE by chlorpyrifos. This deficiency may render the study unacceptable based on incompleteness of expected inclusions. This deficiency is considered a serious omission and is the fault of the persons responsible for designing the study.

-The only subject that showed definite inhibition of RBC AChE did not complete the study after the 48th hour assessments. This subject did not provide blood samples for hematology or clinical chemistry at the scheduled one week post treatment time. There is no way to tell if this subject developed any symptoms to the initial inhibition of RBC AChE that attained 28% decrease at 12 hours. This deficiency may be beyond the control of the laboratory and there is no justification at this time to fault the laboratory for the subject not completing the study.

-The hematology, clinical chemistry, urinalysis and ECG assessments were made one week following the administration of chlorpyrifos. This is considered a poor design. These assessments should have been done during the time of peak effect of chlorpyrifos on AChE. The assessments after one week are of little value since the chlorpyrifos has been cleared from the body and it would be expected that inhibition of AChE would have been partially reversed by this time or that the body adapted to the decreased AChE activity.

Appendix I - Individual Subject Analysis for subjects showing symptoms or variation in AChE

Subject #	Symptoms or Events (a)	RBC AChE Comments	HED's Conclusion
Placebo-Males			
2 (no)	Increased appetite, headache (moderate)- vomited. Starting 47 hrs postdosing, lasting for 4 days, 6 hrs. <i>Possibly related to treatment.</i> "One can coca-cola next day".	Maximum decrease 12% @ 48 hrs. Other values are 89% to 105%. of predose	Since these subjects are in the placebo group, there symptoms or events cannot be due to chlorpyrifos treatment.
8 (yes)	Headache (mild)- Starting 10 hrs postdosing, lasting ~13 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 9% @ 48 hrs. Other values are 94% to 103%.	
10 (yes)	No symptoms or events.	Maximum decrease 14% @ 96 hrs. Other values are 88% to 103%.	
13 (yes)	No symptoms or events.	Maximum decrease 12% @ -10 hrs. Other values are 97% to 112%.	
37 (yes)	Dizzy, feels weak, nausea, vomited, body aches - Starting 2 days, 18 hrs lasting (some symptoms) for up to 2 days 11 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 3% @ 96 hrs. Other values 97% to 107% of predose.	
41 (no)	Itching of insect bite. Starting 2 days 5 hrs, lasting 11 hrs. <i>Unrelated to treatment.</i>	Maximum decrease 7% @ 24 hrs. Other values 95% to 107% of predose.	
45 (no)	Nausea, vomited. Starting 2 days. Lasting ~20 min. <i>Possibly related to treatment.</i>	No decreases. Values are 100 to 110% of the predose.	
46 (no)	Headache (mild). Starting ~ 3 hrs. Lasting ~ 3hrs. <i>Possibly related to treatment.</i>	Maximum decrease 6% @ 2 hrs. Other values 98% to 108% of predose.	
3/12 subjects have headaches. 4/12 thought they received the chlorpyrifos when it was not given. As much as a 14% decrease in RBC AChE can occur within the untreated group over the 7 day period.			
Placebo-Females			
20 (yes)	No symptoms or events.	Maximum decrease 7% at -10 hrs. Other values 94% to 107%.	Since these subjects are in the placebo group,

Subject #	Symptoms or Events (a)	RBC AChE Comments	HED's Conclusion
23 (yes)	No symptoms or events.	Maximum decrease 7% at 144 hrs. Other values 96 to 110%.	there reactions cannot be due to chlorpyrifos treatment.
29 (no)	"hiccough", heartburn. Starting 11 hrs, lasting 11 hrs. Unrelated to treatment.	Maximum decrease 13% at 96 hrs. Other values 95 to 107%.	
32 (no)	No symptoms.	Maximum decrease 17% and sustains from 96 to 144 hrs. Other values 85 to 105%. Subject included to show variation in AChE.	
50 (no)	Headache (mild)- Starting 1 day 21 hrs. lasting 3 hrs. <i>Unlikely related to treatment.</i>	Maximum decrease 12% at 144 hrs. Other values 97 to 108% of predose.	
52 (yes)	Headache (mild), loose stool, nausea, intestinal cramps, vomited. Starting on days 3 and 4, lasting 6-10 hrs (some symptoms). <i>Possibly related to treatment.</i>	Maximum decrease 7% at 24 hrs. Other values 97 to 103% of predose.	
54 (no)	Headache (moderate and mild), nausea. Starting 4-5 hrs, and again at 11 hrs. Lasting 6-7 hrs and 17 hours. <i>Possibly related to treatment.</i>	Maximum decrease 4% at 36 hrs. Other values 97 to 107% of predose.	
60 (yes)	lack of appetite, headache (mild), nausea. Starting at 3-5 hrs or 13 hours. Lasting 5 to 17 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 5% at 4 hrs. Other values 97 to 102% of predose.	
4/12 (33%) females had headaches. 4/12 thought they received the chlorpyrifos when it was not given. As much as 17% reduction in RBC AChE can be attained in the untreated female group.			
0.5 mg/kg Chlorpyrifos-Males			
3 (no)	Increased appetite. Starting 1 day and 23 hrs. Lasting 4 days. <i>Unlikely related to treatment.</i>	Maximum decrease 15% at 48 hrs. Other values 94 to 102% of predose.	<i>Increased appetite is not an expected response to an OP. Not related to treatment Maximum decrease not supported by earlier or later decreases.</i>

Subject #	Symptoms or Events (a)	RBC AChE Comments	HED's Conclusion
5 (yes)	Subject implied excessive salivation was why he thought he received the test material but there were no indications of excessive salivation listed in the events Table.	Maximum decrease 8% at 96 hours. Other values 90 to 108%.	No support from either the symptoms or AChE data to for subjects "yes" response.
9 (no)	Intestinal cramps. Starting 2 days 22 hours. Lasting 15 hrs. <i>Unlikely related to treatment.</i>	Maximum decrease 17% at 96 hrs followed by 11%, 13% and 9% at 120, 144 and 168 hours. Other values Other values were 87% to 105% of predose.	Not related to treatment since onset did not coincide with AChE ↓.
None have headaches. 1/6 thought they received the chlorpyrifos. Note hour 96 had largest decrease in RBC AChE for all but one subject in this group suggesting some assay problem. Since it is hour 96, this should not confound the interpretation of the data.			
0.5 mg/kg Chlorpyrifos-Females			
26 (no)	Nausea. Starting at 1 hr. Lasting 90 min. <i>Probably related to treatment.</i>	Maximum decrease 8% at 96, 144 and 168 hrs. Other values are 93% to 104%.	An initial localized nausea may result from an OP administration in a capsule without concomitant ↓ in AChE. Maximum ↓ in AChE does not coincide with symptoms. Not considered a definite response to chlorpyrifos.
31 (no)	Headache (moderate). Starting 1 day and 3 hrs. Lasting ~2 hrs. <i>Unlikely related to treatment.</i>	Maximum decrease 14% at 96 hrs. Other values were 90% to 104% of predose.	Onset of headache does not coincide with maximum ↓ in AChE occurring much later. Not related to treatment.
36 (yes)	Headache (mild), lightheaded, headache (mild). Starting 1 hr or 6 hrs. Lasting ~2 hrs or ~11 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 9% at 4 and 96 hrs. Other values were 92% to 106%.	Onset of headaches did not coincide with maximum ↓ in AChE.
2/6 (33%) females had headaches. 1/6 thought they received the chlorpyrifos. Maximum decrease in RBC AChE does not exceed the placebo group.			

Subject #	Symptoms or Events (a)	RBC AChE Comments	HED's Conclusion
1.0 mg/kg chlorpyrifos-Males			
1 (yes)	Increased appetite. Starting 1 day and 23 hrs. Lasting 4 days six hrs. <i>Unlikely related to treatment.</i>	Maximum decrease 7% @ 48 hrs. Other values are 95% to 113% of predose.	Symptom not expected of an OP exposure and does not coincide with maximum ↓ AChE.
4 (yes)	Loose stool. Starting 2 days and 15-20 hrs and again on day 4 and 12 hrs. Lasting 1 min. <i>Unlikely related to treatment.</i>	Maximum decrease 15% @ 96 hrs followed by decreases of 8%, 12% and 8% at 120, 144 and 168 hrs. Other values 90% to 107%.	Symptoms do not coincide with maximum ↓ in AChE.
7 (yes)	No symptoms or events.	Maximum decrease 16% at 96 hours. Other values are 91% to 116%.	No support for subjects "yes" response.
11 not listed	Headache (moderate and mild). Starting ~3 hrs and again at ~23 hrs. Lasting ~19 hrs and 1 day 9 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 25% @ 96 hrs. Other values were 89% (at 2 hrs) to 101% (at 4 hrs) of predose.	First headache may be associated with ~11% ↓ in AChE. Second headache not associated with ↓ AChE or maximum decrease.
14 (yes)	Queasy stomach. Starting 24 min. Lasting 10 min. <i>Possibly related to treatment.</i>	Maximum decrease 15% @ 144 hrs. Other values 88% to 99% of predose.	An initial unsettled (queasy) stomach may result from the capsule. Symptom does not coincide with ↓ AChE.
1/6 has a headache. This is less than the placebo group and there is no indication that this subjects headache was more severe. 4/6 thought they received the chlorpyrifos. The maximum decrease in this group was in an individual at 96 hrs postdosing.			
1.0 mg/kg Chlorpyrifos-Females			
24 (no)	Lost voice. Starting ~18 hrs. Lasting 8 hrs.	Maximum decrease 11% @ 168 hrs. Other values 95% to 105% of predose.	Lost voice not an expected response to an OP. Symptom does not coincide with ↓ AChE. Not related to treatment.
25 (yes)	Tired. Starting ~4 hrs. Lasting 1 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 9% @ 96 and 120 hrs. Other values 92%	Symptom does not coincide with maximum ↓ AChE.

Subject #	Symptoms or Events (a)	RBC AChE Comments	HED's Conclusion
		to 102%.	Not related to treatment.
30 (no)	Headache (mild), tired and repeated headaches (mild). Starting 22 min, ~ 2hrs, ~ 10 hrs and ~ 23 hrs. lasting. Up to 8 days and 7 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 8% @ 96 hrs. Other values 95% to 109% of predose/	Onset and duration of symptoms so not coincide with ↓AChE.
33 (no)	Headache (mild). Staring ~ 2hrs. Lasting 4 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 11% @ 144 hrs. Values from 96 hrs to 168 hrs were 89% to 955. Other values were 97% to 111%.	Onset of symptom does not coincide with maximum of any ↓ in AChE.
35 (no)	Back stiffness. Starting 22 min. Lasting ~4 hrs. <i>Unlikely related to treatment.</i>	Maximum decrease 6% @ 168 hrs. Other values 94% to 115%.	Symptom not an expected response to OP treatment and does not coincide with maximum ↓ AChE. Not related to treatment.
2/6 (33%) females had headaches. 1/6 thought they received the chlropyrifos. Maximum decrease dpes nor exceed palcebo group for RBC AChE.			
2.0 mg/kg Chlorpyrifos-Males			
40 (yes)	No symptoms or events.	Maximum decrease 2% at 48 hrs. Other values 99% to 103%.	No support for subjects "yes" response.
47 (no)	Cut on chin. Starting 2 days. Lasting 6 days. <i>Unrelated to treatment.</i>	Maximum decrease 5% at 24 hrs. Other values 98% to 107% of predose.	Not related to treatment.
None have headache. 1/6 thought they received the chlropyrifos. Maximum decrease in group was to 10% for one subject (#48 at 36 hours) and dose not exceed the placebo.			
2.0 mg/kg Chlorpyrifos-Females			
51 (yes)	Headache (mild - moderate). Starting at 47 min and again at 3 hrs 7 min and lasting 2-7 hrs. <i>Possibly/probably related to treatment. One can coca-cola.</i>	Maximum decease 6% at 12 hrs. Other values were 95 to 105% of the predose.	Onset of headache does not coincide with AChE↓. Not related to treatment.
55	Headache (mild). Starting 3 hrs, lasting 19 hrs. <i>Possibly related to</i>	Maximum decrease 5% at 120 hrs. Other values were 97 to	Onset of headache does not coincide with

Subject #	Symptoms or Events (a)	RBC AChE Comments	HED's Conclusion
(no)	<i>treatment.</i>	107% of predose.	AChE↓.
56 not listed	Numbness in upper arms. Starting 42 min. Lasting 2 hrs and 24 min. <i>Possibly related to treatment.</i>	Maximum decrease 28% at 12 hrs. ↓ starts at 8 hours (23%↓),	See special discussion. Numbness does not coincide with AChE ↓.
58 (yes)	No symptoms or events.	Maximum decrease 7% at 12 hrs. Other values are 96% to 101%	No support for subjects "yes" response.
59 (yes)	Tired, headache (mild), chest tightness, shortness of breath, headache . Starting ~ 2hrs, and 10 hrs. Lasting 3-13 hrs. <i>Possibly related to treatment.</i>	Maximum decrease 7% at 12 hrs. Other values were 95% to 104% of predose.	First headache does not coincide with ↓ AChE, the onset of the second headache is two hours before maximum↓ in AChE but ↓ is only 7%. HED does <i>not</i> conclude that the headache is treatment related.
3/6 (50%) females had headaches. Severity of headache not greater than placebo subjects and onset does not coincide with AChE decrease. 3/6 thought they received the chlorpyrifos. One subject has definite inhibition of RBC AChE based on sustained decrease at critical times.			

(a) Only those subjects having reports of symptoms, "events" or responded "yes" (column 1) to the question if they thought they received the test material are listed.

Symptom data are from Appendix 4. Under symptoms, the entry in *italics* refers to the study author's conclusion regarding whether or not the symptom was related to treatment was made before the subjects were identified as to their dose group.

AChE data are from Table 5.1.2 for phase I and Table 9.1.2 for Phase II.