

## DRAFT

### Human Toxicity Studies

#### A. Single-Dose Oral/Dermal Study:

**MRID 00124144. Nolan, R. J.; Rick, D. L.; Freshner, N. L.; *et al.* (1982). Chlorpyrifos: Pharmacokinetics in Human Volunteers Following Single Oral and Dermal Doses. *Toxicology and Applied Pharmacology*. 73, 8-15 (1984).**

In a single-dose human oral/dermal toxicity study with chlorpyrifos (MRID 00124144), 6 Caucasians male subjects (age range 25-50 years old; weight 72 kg to 102 kg) were dosed orally with chlorpyrifos (99.8% a.i.; dissolved in food-grade methylene chloride and placed on a 0.5 g lactose tablet; methylene chloride allowed to evaporate and tablet was swallowed whole with about 100 mL water) after consuming breakfast of cold cereal, sweet roll, fruit juice. There were no control (placebo) subjects, but pre-dose levels (on 3 separate days during 3-week period prior to dose) of plasma and RBC cholinesterase activity were obtained for each subject and used for comparison (Micro-Michel method (1961) for cholinesterase assay]. Dermal doses (5 mg/kg to 5 subjects; 0.5 mg/kg to one subject) were administered (4 weeks after the oral dose) by spreading measured volumes ( $\approx 10$   $\mu\text{L/kg}$ ) of chlorpyrifos dissolved in dipropylene glycol methyl ether (DPGME) on the volar surface ( $\approx 100$   $\text{cm}^2$ ) of the forearm (not covered or occluded). Cholinesterase [ChE] activity [plasma and RBC] was monitored at 2, 6, 12, 24 hours, and 2, 3, 4, 8, 14, 22, 27, and 30 days post dose (micro-Michel method, 1961). Urine was collected 24-48 hours prior to dosing through 120 hours post dosing, with separate collections made for the intervals starting at 0, 6, 12, 24, 36, 48, 60, 72, and 96 hours post dose. Volume and creatinine concentrations of each collection were determined. Urine chlorpyrifos and TCP (principle metabolite 3,5,6-trichloro-2-pyridinol) concentrations were determined also.

The objective of the study was to provide data on the fate of orally and dermally administered chlorpyrifos in the human. The kinetics of chlorpyrifos and its principal metabolite 3, 5, 6-trichloro-2-pyridinal (TCP) were investigated. No clinical signs of toxicity or inhibition of RBC cholinesterase activity were observed following either the oral or dermal dose. Plasma cholinesterase was reduced to 11%-17% of predose levels following the oral dose (peak inhibition within 6-24 hours post dose) but was essentially unchanged following the dermal dose. Blood chlorpyrifos levels were extremely low ( $<30$   $\text{ng/mL}$ ; limit of detection 5  $\text{ng/mL}$ ), and chlorpyrifos was not found in the urine following either route of exposure. TCP blood concentrations peaked 6 hours after oral ingestion (0.93  $\mu\text{g/mL}$ ) and 24 hours after dermal application (0.063  $\mu\text{g/mL}$ ). TCP was cleared from the blood and eliminated in the urine with a half-life of 27 hours following both the oral and dermal doses. Using assumptions of first-order absorption and elimination equations, it was estimated that  $72\pm 11\%$  of the ingested dose and  $0.95 \pm 0.559\%$  (based on the 5 subjects receiving 5 mg/kg dermal dose) and 3.18% (based on one subject receiving 0.5 mg/kg dermal dose) of the dermal dose were absorbed.

#### **Summary of Dosing Regimen for Single-Dose Human Oral/Dermal Toxicity Study for Chlorpyrifos**

There were a total of 6 men. Subject A served as a "pilot" and was given a single oral dose

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(0.5 mg/kg dissolved in methylene chloride). Subject A was also given a single (0.5 mg/kg) dermal dose (dissolved in methylene chloride) four weeks later. Two weeks after the first dermal dose, Subject A was given a second dermal dose (0.5 mg/kg dissolved in DPGME).

The main study consisted of two phases. The five subjects were dosed once orally (0.5 mg/kg) and once dermally (5 mg/kg), thirty days after the oral dose.

Route of exposure	Subject A (n=1)	Subjects B-F (n=5)
Oral	0.5 mg/kg	0.5 mg/kg
Dermal	0.5 mg/kg (methylene chloride)	-
Dermal	0.5 mg/kg (DPGME)	-
Dermal	-	5.0 mg/kg (DPGME)

### Strengths

- Administration using two routes of exposure provides comparative information for single dose.
- 5 subjects/route of exposure
- Blood sampling at multiple time points. Cholinesterase activity was monitored at 2, 6, and 12 hours, and 1, 2, 3, 4, 8, 14, 22, 27, and 30 days post dose, which provides information regarding time to recovery.
- Both plasma and RBC cholinesterase activity monitored
- Chlorpyrifos and metabolite TCP were monitored in blood and urine

### Weaknesses

- not a double-blind study design
- no concurrent control
- only one sex (males)
- only one dose level – no dose-response information for either route of exposure

**Plasma Cholinesterase (Tables 2 and 4):** Following oral exposure, greater than 60% inhibition was observed in 2 of the 5 subjects by 2 hours post dose (first time point monitored), and 4 of the 5 subjects displayed >70% inhibition at 6 hours post dose. Three of the 5 subjects displayed significant plasma cholinesterase inhibition at 2 hours post dose (↓64%, ↓70%, ↓30%), and peak inhibition occurred at 6 hours in one subject (↓88%), at 12 hours in three subjects (↓86%, ↓89%, ↓83%), and at 24 hours in one subject (↓84%). Recovery (< 20% inhibition) was observed by 22-30 days post dose. Following dermal exposure, 2 of the 5 subjects displayed significant plasma cholinesterase inhibition at day 2 (26%) and day 3 (35%)

**RBC Cholinesterase (Tables 3 and 5):** Following oral exposure, one subject displayed RBC cholinesterase inhibition at 2 hours post dose (↓37%), but normal activity thereafter until day 8. Two other subjects displayed RBC cholinesterase inhibition (↓53% and ↓46%) at 4 days post dose. One of these subjects also displayed RBC cholinesterase inhibition on day 30 post dose (↓62%). This latter finding is considered spurious in light of the findings 2 hours latter (following dermal dose), which show comparable levels of activity among all subjects. Following dermal exposure, none of the five subjects displayed RBC cholinesterase inhibition

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at any time point.

<b>Table 2. Plasma Cholinesterase Activity (oral dose)</b>					
<b>Phase/interval</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
<b>Pretest</b>	<b>1.09±0.09</b>	<b>0.87±0.09</b>	<b>1.16±0.12</b>	<b>1.26±0.04</b>	<b>1.42±0.17*</b>
2 hours	-	64*	-	70*	30*
6 hours	12*	<b>88*</b>	72*	84*	78*
12 hours	64*	85*	<b>86</b>	<b>89</b>	<b>83</b>
Day 1	<b>84</b>	86	84	85	80
Day 2	79	77	75	78	78
Day 3	72	74	72	72	76
Day 4	67	64	66	67	68
Day 8	57	48	49	56	54
Day 14	26	17	30	37	27
Day 22	9	22	30	38	28
Day 27	6	-	18	42	15
Day 30	20	8	6	22	21
<b>Chlorpyrifos*</b>	6-12	2-12	1-6	1-6	0-6

**Bolded #s** peak inhibition; numbers are % inhibition from pretest value; \* hours post oral dose chlorpyrifos detected in blood

<b>Table 3. RBC Cholinesterase Activity (oral dose)</b>					
<b>Phase/interval</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
<b>Pretest</b>	<b>0.89±0.18</b>	<b>0.94±0.08</b>	<b>0.91±0.15</b>	<b>0.90±0.17</b>	<b>0.96±0.13</b>
2 hours	+	2	+	37	6
6 hours	+	+	+	+	+
12 hours	+	2	+	+	+
24 hours	+	5	+	+	+
Day 1	+	-	+	+	+
Day 2	+	1	+	-	+
Day 3	+	+	+	11	+
Day 4	53	46	14	+	15
Day 8	+	11	8	27	+
Day 14	17	22	10	27	12
Day 22	X	X	x	X	X
Day 27	8	23	9	+	12
Day 30	62	19	12	+	-

numbers are % inhibition from pretest value; + value greater than pre-dose; - value same as pre-dose; x not used in assessment (reason not evident)

<b>Table 4. Plasma Cholinesterase Activity (dermal dose)</b>					
<b>Phase/interval</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
<b>Pretest</b>	<b>0.87</b>	<b>0.80</b>	<b>1.1</b>	<b>0.99</b>	<b>1.12</b>
▶ 2 hours	13	+	10	12	+
6 hours	+	-	12	14	+

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<b>Table 4. Plasma Cholinesterase Activity (dermal dose)</b>					
<b>Phase/interval</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
12 hours	+	12	14	22	+
Day 1	+	15	18	19	19
Day 2	6	18	18	26	+
Day 3	8	21	18	18	35
Day 4	9	12	16	12	+
Day 6	+	6	8	9	+
Day 8	+	+	-	8	+
Day 9	+	15	9	14	+
<b>Chlorpyrifos*</b>					

♫ activity on day 30 following acute oral dose; ► Time interval is time after dermal dose, which was administered 30 days after oral dose; numbers are % inhibition from pretest; +no inhibition  
**Bolded #s** peak inhibition; \* hours post oral dose chlorpyrifos detected in blood

<b>Table 5. RBC Cholinesterase Activity (dermal dose)</b>					
<b>Phase/interval</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
♫Pretest	<b>0.34♫</b>	<b>0.76</b>	<b>0.80</b>	<b>0.93</b>	<b>0.96</b>
► 2 hours	+	-	+	4	2
6 hours	+	<b>3</b>	+	4	2
12 hours	+	11	+	10	+
Day 1	+	+	+	+	6
Day 2	+	+	+	+	4
Day 3	+	+	+	+	-
Day 4	+	+	8	-	9
Day 6	+	+	+	+	4
Day 8	+	+	+	-	4
Day 9	+	+	+	+	+
<b>Chlorpyrifos*</b>					

♫ activity on day 30 following acute oral dose; ► Time interval is time after dermal dose, which was administered 30 days after oral dose; numbers are % inhibition from pretest; +no inhibition  
**Bolded #s** peak inhibition; \* hours post oral dose chlorpyrifos detected in blood

♫ Subject B value was 0.82 on day 27 and 0.34 on day 30 (% inhibition not >5% using day 27 value)

Chlorpyrifos was detected (sporadically) in the blood of all subjects at various times following the oral (0.006-0.03 µg/mL) and dermal (0.005-0.008 µg/mL) exposures (Table 6). TCP was detected in the blood of all subjects at most time intervals measured following both oral (0.04-1.43 µg/mL) and dermal (0.009-0.122 µg/mL) exposures (Table 7). Following the oral dose, the highest level of TCP in the blood occurred at 2 (C), 4 (E, F), 6 (D), and 24 hours (B) post dose. TCP levels in urine were detected in each subject throughout the monitoring interval (Table 8), with the highest values ranging from 438 to 510µg/mL (oral dose) and 17.7 to 59.3 µg/mL (dermal dose). In all subjects, the highest TCP level in urine was observed at 24 hours.

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<b>Table 6. Blood Chlorpyrifos Levels (µg/mL)</b>					
<b>Hours</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
	<b>Blood Chlorpyrifos Levels (oral dose)</b>				
0	ND	ND	ND	ND	0.009
1	ND	ND	0.006	0.005	0.009
2	ND	0.019	-	0.011	0.015
4	ND	0.009	0.021	0.008	0.009
6	0.012	0.012	0.021	0.008	0.018
8	0.007	0.030	ND	ND	ND
10	0.008	0.006	ND	ND	ND
12	0.028	0.015	ND	ND	ND
	<b>Blood Chlorpyrifos Levels (dermal dose)</b>				
0	0.007	ND	ND	0.010	ND
2	ND	ND	ND	0.008	ND
6	ND	0.005	0.005	ND	ND
10	ND	ND	ND	0.010	0.005
24	ND	ND	0.006	0.007	ND
48	ND	ND	-	-	-

- not analyzed; ND not detected at detection limit of 0.005 µg/mL; **Subject A (ND)**

<b>Table 7. Blood TCP Levels (µg/mL)</b>					
<b>Hours</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
	<b>(oral dose)</b>				
1	ND	0.046	0.009	0.012	0.216
2	0.067	<b>1.18</b>	0.180	1.09	0.996
4	0.229	1.12	0.691	<b>1.19</b>	<b>1.43</b>
6	0.509	0.939	<b>1.35</b>	1.07	1.29
8	0.597	0.907	1.16	0.994	1.16
10	0.813	0.853	0.973	0.810	1.04
12	0.763	0.790	0.934	0.818	1.05
24	<b>0.823</b>	0.598	0.774	0.624	-
48	0.560	0.345	0.540	0.268	0.423
72	0.354	0.199	0.342	0.081	0.215
96	0.194	0.117	0.201	0.060	0.136
	<b>(dermal dose)</b>				
4	ND	0.019	0.018	ND	ND
6	ND	0.067	0.018	0.019	0.013
8	0.028	0.079	0.020	0.024	0.028
10	0.023	0.107	0.037	0.020	0.014
12	0.043	0.102	0.035	0.022	0.019
24	<b>0.070</b>	<b>0.122</b>	<b>0.057</b>	<b>0.036</b>	<b>0.029</b>
48	0.068	0.106	0.056	0.031	0.028
72	0.049	0.080	0.046	0.022	0.019
96	0.032	0.060	0.031	0.016	0.018

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<b>Table 7. Blood TCP Levels (µg/mL)</b>					
<b>Hours</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
	<b>(oral dose)</b>				
144	0.019	0.015	0.017	0.009	ND

- not analyzed; ND not detected at detection limit of 0.005 µg/mL

<b>Table 8. Urine TCP Levels (µg/hour)</b>					
<b>Hours</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
mid-point interval	<b>oral dose</b>				
3	64	231	167	<b>438</b>	<b>451</b>
9	<b>457</b>	<b>510</b>	<b>492</b>	409	427
18	328	269	212	368	273
30	257	229	177	229	184
42	181	140	134	208	175
54	152	121	116	178	129
66	109	96	95	57	91
84	87	65	73	67	64
108	49	38	34	30	36
	<b>dermal dose</b>				
3	1.4	2.2	3.3	ND	ND
9	7.8	35.9	8.8	4.5	10.8
18	21.1	49.1	10.0	15.2	13.2
30	<b>25.9</b>	<b>59.3</b>	17.5	<b>21.0</b>	7.9
42	24.8	43.5	13.4	16.1	<b>55.5</b>
54	17.6	41.4	<b>17.7</b>	13.3	24.2
66	20.6	31.4	12.1	10.7	8.7
84	14.3	24.8	6.4	10.3	16.4
108	9.5	15.4	5.1	5.3	9.9
162	4.5	6.1	4.8	2.4	3.8

ND not detected at detection limit of 0.0025 µg/mL

<b>Table 9. Subject A (pilot) TCP Levels (oral dose)</b>			
<b>Blood</b>		<b>Urine</b>	
<b>Hours</b>	<b>TCP Levels (µg/mL)</b>	<b>Hours</b>	<b>TCP Levels (µg/hr)</b>
1	ND	-	-
2	0.126	-	-
3	-	3	177
4	0.714	-	-
6	0.715	-	-
8	0.650	-	-
9	-	9	268
10	0.606	-	-
12	0.544	-	-
18	-	18	199
24	0.390	-	-

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<b>Table 9. Subject A (pilot) TCP Levels (oral dose)</b>			
<b>Blood</b>		<b>Urine</b>	
<b>Hours</b>	<b>TCP Levels (µg/mL)</b>	<b>Hours</b>	<b>TCP Levels (µg/hr)</b>
30	-	30	131
36	0.270	-	-
42	-	42	119
48	0.185	-	-
54	-	54	73
66	-	66	53
72	0.081	-	-
84	-	84	31
96	0.040	-	-
108	-	108	16

detection limit of 0.005 µg/mL (blood); detection limit of 0.0025 µg/mL (urine)

*ChE methodology.* Micro-Michel method (1961) was used for cholinesterase assay.

The following tables provide the data for all six subjects.

<b>Table 2a. Plasma Cholinesterase Activity (oral dose)</b>						
<b>Phase/interval</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
<b>Pretest</b>	<b>1.29±0.13</b>	<b>1.09±0.09</b>	<b>0.87±0.09</b>	<b>1.16±0.12</b>	<b>1.26±0.04</b>	<b>1.42±0.17*</b>
<b>2 hours</b>	-	-	64*	-	70*	30*
<b>6 hours</b>	57	12*	<b>88*</b>	72*	84*	78*
<b>12 hours</b>	70	64*	85*	<b>86</b>	<b>89</b>	<b>83</b>
<b>Day 1</b>	<b>71</b>	<b>84</b>	86	84	85	80
<b>Day 2</b>	68	79	77	75	78	78
<b>Day 3</b>	38	72	74	72	72	76
<b>Day 4</b>	34	67	64	66	67	68
<b>Day 8</b>	Nd	57	48	49	56	54
<b>Day 14</b>	Nd	26	17	30	37	27
<b>Day 22</b>	-	9	22	30	38	28
<b>Day 27</b>	Nd	6	-	18	42	15
<b>Day 30</b>	Nd	20	8	6	22	21
<b>Chlorpyrifos*</b>	X	6-12	2-12	1-6	1-6	0-6

Pre-test value mean of 2 measurements; - value greater than pre-test; nd no data;

**Bolded #s** peak inhibition; \* hours post oral dose chlorpyrifos detected in blood ; **X not detected at detection limit of 0.005 µg/mL;**

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<b>Table 3a. RBC Cholinesterase Activity (oral dose)</b>						
<b>Phase/interval</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
<b>Pretest</b>	<b>0.92±0.08</b>	<b>0.89±0.18</b>	<b>0.94±0.08</b>	<b>0.91±0.15</b>	<b>0.90±0.17</b>	<b>0.96±0.13</b>
2 hours	11	+	2	+	37	6
6 hours	11	+	+	+	+	+
12 hours	18	+	2	+	+	+
Day 1	16	+	5	+	+	+
Day 2	9	+	1	+	-	+
Day 3	12	+	+	+	11	+
Day 4	7	53	46	14	+	15
Day 8	nd	+	11	8	27	+
Day 14	nd	17	22	10	27	12
Day 22	16	X	X	x	X	X
Day 27	nd	8	23	9	+	12
Day 30	nd	62	19	12	+	-

nd no data; + value greater than pre-dose; - value same as pre-dose; x not used in assessment (reason not evident)

<b>Table 7a. Blood TCP Levels (µg/mL) Following Oral Dose</b>						
<b>Hours</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
1	ND	ND	0.046	0.009	0.012	0.216
2	0.126	0.067	<b>1.18</b>	0.180	1.09	0.996
4	0.714	0.229	1.12	0.691	<b>1.19</b>	<b>1.43</b>
6	<b>0.715</b>	0.509	0.939	<b>1.35</b>	1.07	1.29
8	0.650	0.597	0.907	1.16	0.994	1.16
10	0.606	0.813	0.853	0.973	0.810	1.04
12	0.544	0.763	0.790	0.934	0.818	1.05
24	0.390	<b>0.823</b>	0.598	0.774	0.624	-
48	0.185	0.560	0.345	0.540	0.268	0.423
72	0.081	0.354	0.199	0.342	0.081	0.215
96	0.040	0.194	0.117	0.201	0.060	0.136

**Bolded** highest (peak)

<b>Table 8a. Urine TCP Levels (µg/mL) Following Oral Dose</b>						
<b>Hours</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>
3	177	64	231	167	<b>438</b>	<b>451</b>
9	<b>268</b>	<b>457</b>	<b>510</b>	<b>492</b>	409	427
18	199	328	269	212	368	273
30	131	257	229	177	229	184
42	119	181	140	134	208	175
54	73	152	121	116	178	129
66	53	109	96	95	57	91
84	31	87	65	73	67	64
108	16	49	38	34	30	36

**Bolded** highest (peak)