DATA EVALUATION RECORD – SUPPLEMENTAL – DRAFT 5/26/09

CHLORPYRIFOS
NONGUIDELINE
STUDY TYPE: REPEAT DOSE ORAL TOXICITY - HUMAN
MRID 00095175

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 207-2009

Primary Reviewer:
H.T. Borges, Ph.D., MT(ASCP), D.A.B.T. Signature: _____
Date: _____

Secondary Reviewers:
R.A. Young, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
K. Slusher, M.S. Signature: _____
Date: _____

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EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature**: _____
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** _____
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature**: _____
Risk Assessment Branch III, Health Effects Division (7509P) **Date:** _____

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DATA EVALUATION RECORD – SUPPLEMENTAL

STUDY TYPE: Repeat Dose Oral Toxicity (Human)
Nonguideline

PC CODE: 059101 **DP BARCODE:** None

TEST MATERIAL (PURITY): Dowco 179 (chlorpyrifos; purity not reported)

SYNONYMS: O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl] phosphorothioate

CITATION: Coulaton, R., L. Golberg, T. Griffin. (1972). Safety evaluation of DOWCO 179 in human volunteers. Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY 12208. Report Number not provided. March 1972. MRID 00095175

SPONSOR: Not reported but presumed: The Dow Chemical Company, Midland, MI

EXECUTIVE SUMMARY: In an oral multiple dose study (MRID 00095175), groups of four healthy adult male prisoners received 0.0, 0.014, 0.03, or 0.1 mg/kg bw/day Dowco 179 (purity, stability, and lot number not provided) for 48, 27, 20, and nine days, respectively. No treatment-related effects were found on measured urinalysis, hematology, or clinical chemistry parameters except for depressed plasma cholinesterase (ChE) activity in subjects receiving the test material.

Plasma cholinesterase activity was reduced 46% by Day 6 in subjects treated with 0.10 mg Dowco 179/kg bw/day, which further decreased to 66% by Day 9. Dosing was stopped for the 0.1 mg/kg bw/day group on Day 9 as plasma ChE activity was decreased >20% for all individuals in the dose group. In the 0.03 mg Dowco 179/kg bw/day group, plasma ChE activity was decreased 32% by Day 16 and remained decreased ~30% through Day 20 when treatment was stopped. No significant treatment-related effect on ChE activity was found in subjects of the 0.014 mg/kg bw/day group. Recovery of plasma ChE activity in subjects treated with 0.1 mg/kg bw/day was observed by Day 25 and by Day 21 in subjects of the 0.03 mg/kg bw/day group after cessation of dosing. Erythrocyte acetylcholienesterase activity was not affected at any of the doses.

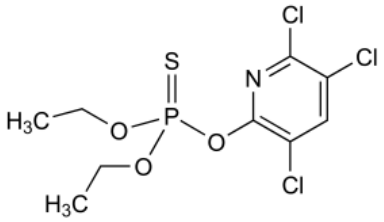
Based on an ~30% decrease in plasma cholinesterase activity by Day 16, the LOAEL for Dowco 179 was 0.03 mg/kg bw/day. The corresponding NOAEL was 0.014 mg/kg bw/day.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Dowco 179

Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Compound Stability:	Not reported
CAS # for TGAI:	2921-88-2
Structure::	

2. Vehicle: Tablet (not further described) provided with the breakfast meal

3. Human Subjects:

Number:	Sixteen
Race:	Not reported
Sex:	Male
Age:	Not reported
Weight:	Not reported
Source:	Volunteers from the Clinton Correctional Facility, Dannemora, NY
Physical Examination:	Before the start of the study, each volunteer was given a thorough physical examination that included a chest X-ray, electrocardiogram, urinalysis, and hematology and clinical chemistry parameters.

B. STUDY DESIGN:

1. Study date: Not reported

2. Study subjects: Sixteen male volunteers from the Clinton Correctional Facility were divided into groups of four and assigned to the groups and duration of treatment shown in Table 1.

Table 1. Study design

Subject #	Dose (mg/kg bw/day)	Duration of exposure
2, 4, 9, 16	0.0	7 weeks
7, 8, 14, 15	0.014	27 days
10, 11,12,13	0.03	20 days
1, 3, 5, 6	0.10	9 days

3. Dose selection: A dose selection rationale was not provided.

4. Dose preparation and analysis: Other than the doses being dispensed onto a tablet at the concentrations shown in Table 1 and provided with the breakfast meal, no other dosing information was provided.

Results:
Homogeneity analysis: Not reported
Stability analysis: Not reported
Concentration analysis: Not provided

5. **Statistics:** Statistical analyses for hematology, clinical chemistry, and urinalysis were not reported. Plasma and RBC cholinesterase activity were analyzed by three-way randomized factorial ANOVA.

C. METHODS:

1. **Observations:** It is assumed the test subjects were observed daily at the time of treatment and/or blood collection; however no specific mention is made in the study report.

2. **Body weight:** Although the doses were administered on a kg bw/day basis, body weight data were not provided in the study report.

4. **Hematology and clinical chemistry:** Blood was collected for hematology and clinical chemistry analyses before treatment and on days 7, 15, 22, 28, 35, 42, and 49 for control subjects; days 6, 13, 20, and 27 for subjects treated with 0.014 mg/kg bw/day; days 7, 15, and 22 and day 6 post-treatment for subjects treated with 0.03 mg/kg bw/day; and day 8 of treatment and days 5, 11, and 18 post-treatment for subjects treated with 0.1 mg/kg bw/day. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)		Mean corpusc. HGB conc.(MCHC)
	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
	Platelet count		Reticulocyte count
	Blood clotting measurements	X	Basophils
	(Thromboplastin time)	X	Eosinophils
	(Clotting time)	X	Neutophils
	(Prothrombin time)	X	Lymohocytes
		X	Monocytes

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin
	Chloride		Creatinine
	Magnesium	X	Urea nitrogen
X	Phosphorus	X	Total Cholesterol
	Potassium		Globulins
	Sodium	X	Glucose
X	**ENZYMES**	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total protein (TP)
X	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase	X	Uric Acid
X	Lactic acid dehydrogenase (LDH)		
	Alanine aminotransferase (ALT/ SGPT)		
X	Aspartate aminotransferase (AST/ SGOT)		
	Gamma glutamyl transferase (GGT)		
	Sorbitol dehydrogenase		
	Glutamate dehydrogenase		

5. **Urinalysis:** Urine was collected weekly during the study and analyzed for specific gravity, color, clarity, pH, and microscopic analysis. Samples of the urine were retained for determination of Dowco 179 and metabolic products.

6. **Cholinesterase activity**: Plasma and RBC cholinesterase (ChE) activity was measured by the method of Nabb and Whitfield (Arch. Environ. Health (1967) 15: 147) with acetylcholine perchlorate as the substrate followed by titration to pH 8.0 with sodium hydroxide. ChE activity was expressed as µmoles acetate/min/mL. Plasma and RBC ChE activity was monitored every 2-3 days during treatment (control: 1, 3, 6, 10, 13, 16, 18, 20, 23, 27, 30, 34, 37, 41, 44, 48 days; 0.014 mg/kg bw/day: 3, 6, 9, 13, 16, 20, 23, 27 days; 0.03 mg/kg bw/day: 1, 3, 6, 13, 16, 18, 20 days; 0.10 mg/kg bw/day: 1, 3, 6, 9 days). After the treatment period, plasma and RBC ChE activity were monitored on the following until activities returned to pre-treatment levels: **control**: days 3 and 7; **0.10 mg/kg bw/day**: days 2, 4, 7, 11, 14, 18, 21, 25, 28, 32, and 35; **0.03 mg/kg bw/day**: days 2, 6, 9, 13, 20, 23, 27, and 30; **and 0.014 mg/kg bw/day**: days 3 and 7.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were reported, however one subject treated with 0.10 mg/kg bw/day Dowco 179 complained of a runny nose, blurred vision, and faintness on the final day of treatment. The subject was treated for a cold and was asymptomatic by the end of the day.

2. **Mortality:** All subjects survived the study.

B. BLOOD ANALYSES:

1. **Hematology:** While individual data were provided, summary data of measured hematological parameters were not provided. Review of the individual data indicates there were no treatment-related effects.

2. **Clinical Chemistry:** While individual data were provided, summary data of measured clinical chemistry parameters were not provided. Review of the individual data indicates there were no treatment-related effects.

F. URINALYSIS: Urinalysis data were not provided. The study author stated that no treatment-related effects were found. Routine urinalysis which included specific gravity, color, turbidity, pH and determination of cellular content were completely negative of treatment related effects for all individuals throughout the study. Urine samples were examined for the presence of Dowco 179, its oxygen analog and its metabolite 3,5,6-trichloro-2-pyridinol and were not detected in any of the samples. Details of the analysis and the limit of detection were not provided except for a reference to the method used.

G. CHOLINESTERASE ACTIVITY:

1. **Plasma:** As shown in Table 2, plasma ChE activity was reduced 46% by Day 6 in the 0.10 mg/kg bw/day dose group, and further decreased to 66% of pretreatment activity by Day 9. The study authors reported that dosing was stopped for the 0.1 mg/kg bw/day group on Day 9 because plasma ChE was decreased >20% for all individuals in this dose group. In the 0.03 mg/kg bw/day group, plasma ChE activity was decreased 32% of pretreatment activity by Day 16 and remained decreased ~30% through Day 20 when treatment was stopped. No significant treatment-related effect was found in subjects of the 0.014 mg/kg bw/day group. Recovery of plasma ChE activity in subjects treated with 0.1 mg/kg bw/day was observed by Day 25 and by Day 21 in subjects of the 0.03 mg/kg bw/day group after cessation of dosing.

Table 2. Average percent change of plasma cholinesterase relative to pre-treatment activity in subjects receiving Dowco 179 daily

Dose (mg/kg bw/day)	Day of treatment								
	1	**3**	**6**	**10**	**13**	**16**	**19**	**20**	**27**
Control	16 ± 33.7	9 ± 21.1	11 ± 6.0	20 ± 10.8	2 ± 11.0	2 ± 12.9	5 ± 15.1	7 ± 7.7	7 ± 11.0
0.014[a]	-	6 ± 9.4	6 ± 12.4	-10 ± 15.8	-20* ± 7.0	-19* ± 18.0	-	-8 ± 14	-6 ± 18.9
0.03[b]	-5 ± 6.8	-9 ± 11.4	-15* ± 17.9	-2 ± 29.3	-15* ± 18.9	**-32*** ± 15.4	**-33*** ± 15.1	**-29*** ± 15.6	-
0.10[c]	-11 ± 21.9	-10 ± 22.1	**-46*** ± 22.1	**-66*** ± 20.9	-	-	-	-	-
Dose (mg/kg bw/day)	**Day of recovery**								
	2	4	7	11	14	18	21	25	28
Control[d]	14 ± 50.9	-	10 ± 48.2	-	-	-	-	-	-
0.014[d]	13 ± 57.3	-	14 ± 56.4	-	-	-	-	-	-
0.03[e]	-25 ± 15.0	-	**-22** ± 15.9	**-24** ± 19.7	**-30** ± 25.2	-	-6 ± 4.8	-7 ± 9.8	-10 ± 14.2
0.10	**60*** ± 21.1	**-52** ± 21.3	**-43*** ± 22.1	**-28** ± 20.2	**35** ± 15.2	**-40** ± 12.1	**-27** ± 18.0	-2 ± 19.7	2 ± 14.7

Results and statistics calculated by reviewer from data in Tables 33a and 33b on pages 39-40 of MRID 00095175
Bolded results indicate >20% ChE acivity depression
[a] Actual days activity measured = 3, 6, 9, 13, 16, 20, 23, and 27
[b] Actual days activity measured = 1, 3, 6, 10, 13, 16, 18, and 20
[c] Actual days activity measured = 1, 3, 6, and 9
[d] Actual days activity measured = 3 and 7
[e] Actual days activity measured = 2, 6, 9, 13, 20, 23, and 27
* = $p \leq 0.05$

2. RBC: As shown in Table 3, Dowco 179 did not decrease the activity of RBC ChE during the treatment period.

Table 3. Average percent change of RBC cholinesterase activity relative to pre-treatment activity in subjects receiving Dowco 179 daily

Dose (mg/kg bw/day)	Day of treatment								
	1	3	6	10	13	16	19	20	27
Control	-6 ± 5.8	4 ± 5.4	11 ± 3.7	14 ± 5.7	12 ± 4.1	5 ± 4.5	5 ± 2.5	3 ± 2.8	20 ± 10.3
0.014[a]	-	0 ± 7.1	3 ± 11.3	-7 ± 9.9	-9 ± 9.1	1 ± 6.3	-	1 ± 6.9	2 ± 8.8
0.03[b]	-1 ± 3.6	6 ± 5.7	9 ± 5.3	9 ± 6.1	11 ± 2.1	4 ± 2.7	5 ± 1.9	1 ± 4.6	-
0.10[c]	-1 ± 6.9	19 ± 4.7	12 ± 7.0	6 ± 3.9	-	-	-	-	-

Results calculated by reviewer from data Tables 34a and 34b on pages 41-42 of MRID 00095175
[a] Actual days activity measured = 3, 6, 9, 13, 16, 20, 23, and 27
[b] Actual days activity measured = 1, 3, 6, 10, 13, 16, 18, and 20
[c] Actual days activity measured = 1, 3, 6, and 9

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The Study investigator concluded that ingestion of Dowco 179 at 0.03 mg/kg/day by humans for 21 days was a threshold level for measured changes in plasma ChE activity with no significant toxicological effect. The plasma ChE activity in this group averaged 87% of the concurrent controls when compared on day to day basis and this was considered not statistically significantly different from the control. Ingestion of 0.1 mg/kg/day of Dowco 179 resulted in marked plasma ChE depression which resulted in termination of the dosing after 9 days. The RBC ChE activity was not affected in any of the groups tested.

B. REVIEWER COMMENTS:

Groups of four male subjects were treated with 0.0, 0.014, 0.03, or 0.1 mg Dowco 179/kg bw/day up to 27 days. No treatment-related effects were found on measured urinalysis, hematology, or clinical chemistry parameters in subjects receiving the test material. Data on the concentration of Dowco 179 and its metabolites in the urine were not provided. Plasma ChE activity was reduced to 46% of pre-treatment activity by Day 6 in subjects treated with 0.10 mg Dowco 179/kg bw/day, which further decreased to 66% of pretreatment activity by Day 9. The study authors reported that dosing was stopped for the 0.1 mg/kg bw/day group on Day 9 because plasma ChE was decreased >20% for all individuals in this dose group. In the 0.03 mg Dowco 179/kg bw/day group, plasma CHE activity was decreased to 32% of pretreatment activity by Day 16 and remained decreased ~30% through Day 20 when treatment was stopped. No significant treatment-related effect on ChE activity was found in subjects of the 0.014 mg/kg bw/day group. Recovery of plasma ChE activity in subjects treated with 0.1 mg/kg bw/day was observed by Day 25 and by Day 21 in subjects of the 0.03 mg/kg bw/day group after cessation of dosing.

Based on an ~30% decrease in plasma cholinesterase activity by Day 16, the LOAEL for the study was 0.03 mg/kg bw/day. The corresponding NOAEL was 0.014 mg/kg bw/day.

C. STUDY STRENGHTS AND WEAKNESSES:

Strengths:

- Concurrent control (placebo)
- Three dose levels (provides quality dose-response information)
- Plasma and RBC ChE activity were monitored
- Blood sampling at multiple time points. ChE activity was monitored on: 1, 3, 6, 10, 13, 16, 18, 20, 23, 27, 30, 34, 37, 41, 44, 48 days (control); 3, 6, 9, 13, 16, 20, 23, 27 days (0.014 mg/kg bw/day); 1, 3, 6, 13, 16, 18, 20 days (0.03 mg/kg bw/day); and 1, 3, 6, 9 days (0.10 mg/kg bw/day).
- Recovery phase
- Chlorpyrifos and metabolites (oxon and TCP) were monitored in urine. None of the compounds were detected in any sample (limit of detection was not provided)

Weaknesses:

- Only one sex (males) used (age and weight ranges were not provided).
- The purity, lot number, and stability of the test material were not provided.
- The study was conducted at a correctional facility.
- Each dose was given for a different duration without a clear rationale (i.e. Control for seven weeks, 0.014 mg/kg bw/day for 27 days, 0.03 mg/kg bw/day for 20 days and 0.1 mg/kg bw/day for nine days).
- ChE activity measurements were not done every day post dose.
- The time after dose when samples were collected for ChE activity was not reported.
- The time of urine collection relative to time of dose was not provided.
- Limit of detection of chlorpyrifos and metabolites was not provided.