Data Evaluation Record

1. Chemical: Dylox Technical (Trichlorfon)
   Shaughnessy No.:057901

2. Test Material: Trichlorfon [Dimethyl(2,2,2-trichloro-1-hydroethyl)phosphonate], 99.8%, batch #203-0021, Lot #1542102252 (CAS #52-68-6); a white crystalline solid

3. Study type: Avian Reproduction (71-4)
   Test Species: Mallard Duck Anas platyrhynchos

4. Study ID: Pedersen, C.A. and S.M. Thompson. 1993. Effects of DYLOX Technical on Mallard Reproduction. Performed by Bio-Life Associates, Ltd., Neillsville, WI for Miles Incorporated, Agriculture Division, P.O. Box 4913, Kansas City, MO 64120. Report #106226. **MRID #430196-01.**

5. Reviewed by: Kathryn V. Montague, M.S.
   Biologist
   EEB/EFED
   Signature: Kathryn V. Montague
   Date: 3/28/94

6. Approved by: Norm Cook
   Head, Section II
   EEB/EFED
   Signature: Norman J. Cook
   Date: 03.28.94

7. Conclusions: The study is scientifically sound and is classified as core. The NOEC for eggshell thickness and percent viable embryos was 27 ppm (LOEC = 78 ppm). The NOEC for all other parameters was 78 ppm (LOEC = 235 ppm).

8. Recommendations: N/A

9. Background information: This study was submitted under Section 6(a)(2) as new information, and was reviewed as a submission toward fulfillment of Guideline 71-4 in support of reregistration for trichlorfon.

10. Discussion of Individual Tests: N/A

11. Materials and Methods:

a. Test animals: Twenty-week-old pen-reared mallards were obtained from Whistling Wings, Inc., Hanover, IL. The birds were all from the same hatch and in their first breeding season. They were acclimated to laboratory conditions for 24 days prior to the initiation of the study.

b. Test system: The birds were housed in 61 x 121.9 x 61 cm galvanized steel mesh pens containing one male and 1 female mallard each. The birds were given fresh feed weekly and ad libitum water, including a small bowl in which they could submerge their nares. The average daily temperature in the

study room was 68°F, and the average relative humidity was 71%.

c. Study design: The birds were maintained for 8 weeks on treated feed on a 7-hr light/17-hr dark photoperiod. At the end of the eight week, the light was increased to 17 hours per day, and this photoperiod was maintained for the rest of the study. The birds were presented with treated feed (Dylox-treated Purina custom gamebird Layena) until the end of week 10. Dosing levels were: control (corn oil and acetone), 30, 90 and 270 ppm a.i. Dylox (nominal). Test diets were prepared fresh weekly and stored in a freezer. Stability testing was performed for 1 week under environmental conditions of the animal room during a previous pilot study. Three samples were taken from the top, middle and bottom of the 30 and 270 ppm diets immediately after preparation on week 1 for homogeneity testing. Samples from the control and 90 ppm mixes during week 1 and from all levels during weeks 5, 10, 15 and 20 were taken to verify concentration of Dylox.

Each treatment group consisted of 16 pairs of mallards. The adults were observed once a day throughout the study. Body weight was measured biweekly for the first 10 weeks and at test termination. Feed consumption was measured biweekly for weeks 2 - 21. Egg laying began in week 11. Eggs were collected daily; the size of each egg was recorded, the eggs were candled and any cracked, broken, soft-shelled or membranous eggs were noted and discarded. The eggs were held in an egg storage room at 64-67°F, 80% RH and turned once a day for 7 days. At the end of each 7-day collection period (or "hatch"), eggs were moved from the holding room to an incubator, where they were automatically turned every 2 hours. Eggs were candled and misted on days 14 and 21. On day 23 of incubation, eggs were placed in hatching trays and misted prior to hatch. On incubation days 27-28, hatchlings, unhatched eggs and shells were recovered. Hatchling body weight was recorded and the hatchlings were placed in brooders for 14 days, during which they were fed untreated feed (Purina custom gamebird Startena) and provided water ad libitum. On day 14, duckling body weight was recorded and the birds were sacrificed.

Eggs collected on the first day of hatches A, C, E, G, I, and K were tested for eggshell thickness at the equatorial circumference, 3 times per egg. They were broken, washed and allowed to air-dry for 48-hours at room temperature. An Ames pocket thickness measure was used to measure shells to the nearest 0.01 mm.

d. Statistics: The following parameters were analyzed: Adult body weight; adult feed consumption;, egg laid, per hen; eggs cracked or broken, as a percentage of total laid and per hen; eggs set, per hen; viable day 14 embryos, as a percentage of

eggs set per hen; viable 3-week embryos, as percentage of number viable day 14 embryos per hen; normal hatchlings, per hen and as a percentage of 3-week embryos; hatchling body weight; number of 14-day survivors, as percent of hatchlings per hen; survivor body weight; and eggshell thickness. All statistical analyses were performed by Sebaugh's Information Services as a consultant to Bio-Life Associates. The SAS system was used to perform the statistical analyses.

12. Reported Results: The mean measured concentrations of Dylox in test feed, determined from sampling, were 0.0 (control), 27.3, 77.8 and 235 ppm a.i. The diets were determined to be homogeneous and stable for 7 days under conditions of the animal room.

There were 5 adult mortalities during the test period, 1 in the control group and 4 at the 235 ppm level. The control bird which died exhibited no symptoms prior to death, while those in the 235 ppm group exhibited clinical signs of toxicity prior to death. The mates of all birds that died were sacrificed, and both birds in each case were necropsied. All 5 mortalities had abnormal findings, as well as the mate of 1 of the 235 ppm mortalities. Necropsies were also performed on 50% of the surviving birds in each test level. Abnormalities were found in 4 control birds, 1 27 ppm bird and 5 235 ppm birds. The findings were not consistent, however, and were not attributed to the test material.

Adult body weight was decreased for weeks 2, 4, 8 and 21 in the 235 pm males and for week 21 in the 235 ppm females. Feed consumption was decreased at the 235 pp level for weeks 2, 12, 16 and 18, and overall. Dylox at 235 ppm may have acted as a repellant.

Clinical signs of toxicity were noted in 2 males and 1 female at 235 ppm. These signs included: lethargy, chalky excreta, sitting in corner, and stumbling and fluttering when prodded. These 3 birds died after the clinical symptoms were noted.

There was a decrease in the number of eggs laid per hen at 235 ppm.

The number of viable embryos (% of eggs set) was decreased at 78 and 235 ppm.

The mean day 1 hatchling body weights were reduced at 235 ppm.

The overall mean eggshell thickness was reduced at 235 ppm.

13. Study Author's Conclusions/Quality Assurance Report: Based on the above results, the NOEC for percent viable embryos was 27 ppm (LOEC = 78 ppm). The NOEC for all other parameters examined was 78 ppm (LOEC = 235 ppm).

Quality Assurance and Good Laboratory Practice statements were included in the report.

14. Reviewer's Discussion and Interpretation of the Results:
a. Test Procedure: The test design and procedure were scientifically sound and in accordance with Guidelines recommendations.

b. Statistical Analysis: All statistics were verified using the SAS system and EPA's TOXSTAT computer program. All values were in agreement with the reported results, with the exception of eggshell thickness data. William's test showed a significant reduction in eggshell thickness at 78 ppm, as well as at 235 ppm (see attached printout). The reviewer also analyzed adult body weight change using TOXSTAT, which showed a significant increase in weight loss for males at 78 and 235 ppm for weeks 0 - 2 (Dunnett's and William's tests) and weeks 6 - 8 (William's test), and for females at 235 ppm for weeks 0 -2 (Dunnett's and William's tests).

c. Discussion/Results: The study is scientifically sound and is classified as core.

d. Adequacy of the study:
(1) Classification: Core.
(2) Rationale: N/A
(3) Repairability: N/A

4	235 ppm	6	0.3070	0.3070
4	235 ppm	7	0.3000	0.3000
4	235 ppm	8	0.3200	0.3200
4	235 ppm	9	0.3770	0.3770

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	13	0.358	0.458	0.396
2	27 ppm	16	0.338	0.432	0.387
3	78 ppm	16	0.281	0.411	0.371
4	235 ppm	9	0.278	0.387	0.332

Trichlorofon mallard repro.--eggshell thickness (means)
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.001	0.030	0.008
2	27 ppm	0.001	0.024	0.006
3	78 ppm	0.001	0.037	0.009
4	235 ppm	0.001	0.036	0.012

Trichlorofon mallard repro.--eggshell thickness (means)
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	0.0245	0.0082	8.200
Within (Error)	50	0.0498	0.0010	
Total	53	0.0744		

Critical F value = 2.84 (0.05,3,40)
Since F > Critical F REJECT Ho:All groups equal

Trichlorofon mallard repro.--eggshell thickness (means)
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DUNNETTS TEST

****** WARNING ******

This data set has unequal replicates. The Bonferroni T-test should be used instead of the Dunnetts test.

Trichlorofon mallard repro.--eggshell thickness (means)
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.396	0.396		
2	27 ppm	0.387	0.387	0.739	
3	78 ppm	0.371	0.371	2.094	
4	235 ppm	0.332	0.332	4.624	*

Dunnett table value = 2.13 (1 Tailed Value, P=0.05, df=40,3)

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	13			
2	27 ppm	16	0.025	6.4	0.009
3	78 ppm	16	0.025	6.4	0.025
4	235 ppm	9	0.029	7.4	0.063

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	0.0245	0.0082	8.200
Within (Error)	50	0.0498	0.0010	

Total	53	0.0744

Critical F value = 2.84 (0.05,3,40)
Since F > Critical F REJECT Ho:All groups equal

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.396	0.396		
2	27 ppm	0.387	0.387	0.739	
3	78 ppm	0.371	0.371	2.094	
4	235 ppm	0.332	0.332	4.624	*

Bonferroni T table value = 2.19 (1 Tailed Value, P=0.05, df=50,3)

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	13			
2	27 ppm	16	0.026	6.5	0.009
3	78 ppm	16	0.026	6.5	0.025
4	235 ppm	9	0.030	7.6	0.063

Trichlorofon mallard repro.--eggshell thickness (means)
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	0.0245	0.0082	8.200
Within (Error)	50	0.0498	0.0010	
Total	53	0.0744		

Critical F value = 2.84 (0.05,3,40)
Since F > Critical F REJECT Ho:All groups equal

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 4 3 2 1
4	235 ppm	0.332	0.332	\
3	78 ppm	0.371	0.371	* \
2	27 ppm	0.387	0.387	* . \
1	control	0.396	0.396	* . . \

* = significant difference (p=0.05) . = no significant difference
Tukey value (4,50) = 3.79 s = 0.001

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	13	0.396	0.396	0.396
2	27 ppm	16	0.387	0.387	0.387
3	78 ppm	16	0.371	0.371	0.371
4	235 ppm	9	0.332	0.332	0.332

Trichlorofon mallard repro.--eggshell thickness (means)
File: b:malegthk.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	0.396				
27 ppm	0.387	0.739		1.68	k= 1, v=50
78 ppm	0.371	2.094	*	1.76	k= 2, v=50
235 ppm	0.332	4.624	*	1.79	k= 3, v=50

s = 0.032
Note: df used for table values are approximate when v > 20.