2-EEB-27-(F)

Reviewer: Conchi Rodriguez 3/26/93

TRID No. 470257-023

DATA EVALUATION RECORD

1. **CHEMICAL:** Metolachlor.
Shaughnessey No. 108801.

2. **TEST MATERIAL:** CGA-24705; technical code FL-760037; ARS No. 1674/77; purity 97.4%; a viscous, light amber liquid.

3. **STUDY TYPE:** 72-5. Fish Life-Cycle Toxicity Test. Species Tested: Fathead Minnow (*Pimephales promelas*).

4. **CITATION:** Anonymous. 1978. Chronic Toxicity of CGA-24705 to the Fathead Minnow (*Pimephales promelas*). Report No. BW-78-11-341. Prepared by EG&G, Bionomics, Wareham, MA. Submitted by Ciba-Giegy Corporation, Greensboro, NC. EPA TRID No. 470257-023.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 3/24/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 3/24/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study is not scientifically sound because both dilution water and solvent control solutions were contaminated with the test material and complete mortality occurred in one of the solvent control replicates. In addition, raw water quality, analytical, and biological data were not submitted for review. Based on the summarized results in the report and the reviewer's analysis of the survival and reproduction data, the MATC was >0.78 and <1.6 mg/l mean measured concentrations (geometric mean MATC = 1.1 mg/l).

8. **RECOMMENDATIONS:** See Section 14.D.(3).

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9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Fathead minnow (*Pimephales promelas*) embryos were obtained from the EPA Water Quality Laboratory, Duluth, MN.

B. **Test System:** A proportional diluter system with a 50% dilution factor was used. The glass test aquaria measured 90 x 30 x 30 cm. Each aquarium was subdivided by stainless steel screens into a spawning area and two larval growth chambers (30 x 12 x 25 cm). A flow splitting chamber over each aquarium delivered 700 ml of test solution to the spawning area and 150 ml to each of the growth chambers. The water level in each aquarium was maintained at 15 cm by a standpipe. The flow rate provided 6-8 volume turnovers per day.

Embryo incubation cups were 5-cm diameter glass jars with stainless steel screen bottoms. The cups were oscillated in the test solution using a rocker arm driven by a 2-rpm motor. Five spawning sites constructed of halved 7.5-cm lengths of cement-asbestos drain pipe (10-cm diameter) were placed in each spawning compartment.

The test temperature was maintained by placing the aquaria in heated water baths. The test system was maintained under an EPA-recommended photoperiod schedule with adjustments in daylength occurring on the first and fifteenth day of each month. The entire test system was enclosed in black plastic curtains to prevent disturbance and minimize the influence of laboratory lighting on the intended photoperiod.

The dilution water was well water drawn from a 125-m deep well and was aerated and stored in a concrete reservoir prior to use.

Stock solutions were prepared on a weight to volume basis every two weeks by dissolving 71.0436 g of test material in acetone to a final volume of 250 ml. A gas tight syringe and mechanical injector introduced the stock into the system. The syringe was refilled every 3-4 days.

C. **Dosage:** Two-hundred and sixty-six-day, flow-through, life-cycle toxicity test. Preliminary acute and sub-acute exposures were performed to identify the concentration range to be used in the life cycle test. Based on these results, five nominal concentrations (0.25, 0.5, 1.0, 2.0, and 4.0 mg/l), a solvent control (14 µl acetone/l), and a dilution water control were used. The solvent concentration in the solvent control was slightly higher than the concentration (10 µl/l) used in the highest test concentration.

D. **Design:** Each treatment level and control was duplicated. One-hundred fathead minnow embryos were divided between two incubation cups in each aquarium.

Each day until hatching was complete (day 4), embryos in each cup were counted and dead embryos were removed. Percentage hatching success was calculated for each duplicate aquarium.

Newly-hatched fry were fed brine shrimp nauplii three times daily for the first 30 days. Between 30 and 60 days, the fish were fed brine shrimp nauplii twice daily and frozen brine shrimp once daily. After the 60-day old fish were transferred to the spawning chamber, they were fed the frozen brine shrimp twice daily and supplemented with high-protein trout granules and live *Daphnia magna*.

Twenty newly-hatched larvae were randomly selected from each incubation cup and placed into their respective growth chambers. After 35 and 64 days of exposure, each group was photographed over a grid for length determinations. Percentage survival was also determined at these intervals. On day 64, the two larval groups in each aquarium were combined and fifteen fish were selected to continue the exposure in the spawning chamber.

After approximately 181 days of exposure, the number of fish in each aquarium was reduced to establish a sex ratio of three males to six females, if possible. Four aquaria had no surviving fish and several aquaria had fewer than six females. Spawning substrates were checked daily for the presence of eggs. For each spawning group, the number of eggs spawned and incubated was recorded. Fifty embryos from a maximum of eleven spawns in each aquarium were incubated to determine hatching success. Eggs spawned by control

fish were incubated in aquaria where no first generation fish survived.

Twenty fry from each of two egg groups with >80% hatching success were placed into their respective growth chambers in each aquarium. After 34 days of exposure, each larval group was photographed and wet weighed.

Exposure of F_0 parental spawners was terminated when spawning had virtually ceased (day 266). At test termination, each fish was individually measured and weighed and internally examined to verify sex and gonadal condition.

During the test, the test aquaria were siphoned at least once weekly and brushed periodically to remove algae.

The dissolved oxygen concentration (DO) and temperature were measured weekly in each aquaria on a rotating basis. Hardness and pH were measured monthly in all aquaria. Acidity, alkalinity, and conductivity were measured in all aquaria.

Water samples were taken from each duplicate aquarium weekly and analyzed for CGA-24705 using gas chromatography.

E. **Statistics:** For statistical evaluation, data for survival, growth, and reproduction were subjected to analysis of variance (ANOVA; p=0.05). Percentage survival and percentage hatching success were arcsine square-root transformed prior to analysis. If treatment effect was indicated, treatment means were compared to control means using Dunnett's test.

12. **REPORTED RESULTS:** The mean measured concentrations for the exposure period were 0.20, 0.37, 0.78, 1.6, and 3.4 mg/l (Table 3, attached). These values were 74-85% of nominal concentrations.

Mean hatching success (%) of F_0 embryos in the treatments was similar to that of the dilution water control and solvent control (Table 4, attached). Thirty days after hatching, no F_0 larvae had survived exposure to 3.4 mg/l mean measured concentration and survival at the 1.6 mg/l test level was significantly reduced (Table 4, attached). Virtually all larval mortality occurred during the fourth week of exposure. After 64 days exposure, survival of fry

remained unaffected by test concentrations ≤0.78 mg/l. Growth (total length) of F_0 larvae surviving after 35 and 64 days exposure was unaffected by concentrations ≤1.6 mg/l.

Survival of solvent control fish and dilution water control fish was comparable during the initial 35 days of the exposure. Between days 54 and 60, all fish in solvent control replicate A died due to a suspected but unconfirmed microbial infection. On day 64, fifteen fish from solvent control replicate B were transferred to replicate A. These fish died between days 67 and 70 when water flow to the aquarium was interrupted by a break in the delivery tubing.

On day 181, the fish were removed from the aquaria for sex determinations. Two fish exposed to 0.78 mg/l were found to have severe spinal deformities. It was not possible to establish the desired sex ratio in many aquaria due to insufficient numbers of fish (Table 5, attached). In these aquaria, all female fish were retained and three male fish were selected at random. "The inability to establish the desired number of six females in all aquaria did not impact the assessment of reproduction which was based on number of spawns and eggs per female." Only two mortalities occurred among the F_0 fish between day 181 and test termination.

By test termination, survival of spawning adults was unaffected by test concentrations ≤0.78 mg/l. Individual total lengths and wet weights of male and female fish were not significantly affected by exposure to test concentrations ≤1.6 mg/l (Table 6, attached).

The reproduction data were summarized in Table 7 (attached). The number of spawns per female and the number of eggs spawned per female among fish exposed to concentrations ≤1.6 mg/l were not significantly different from the control.

Hatching success of F_1 embryos spawned from fish exposed to test concentrations ≤1.6 mg/l was comparable to the control (Table 8, attached). Embryos spawned in the control but incubated at 3.4 mg/l hatched successfully, but after 34 days exposure, their survival was significantly reduced. As in the F_0 generation, most mortality in the 3.4 mg/l group occurred during the fourth week of exposure. Length and weight of F_1 larvae from the 3.4 mg/l exposure group were significantly lower than those of the control. Length of larvae exposed to 0.37 mg/l was also significantly lower than the control. "A significant difference in length between control and 0.37 mg/l was not indicated when the actual difference and the critical value were rounded to the

nearest millimeter to reflect the accuracy of measuring the fish."

Dissolved oxygen concentrations were 4.5-9.4 mg/l and pH ranged from 6.8 to 7.4 during the study. The conductivity was 130-170 µmhos/cm. The alkalinity and hardness were 25-30 and 21-44 mg/l as $CaCO_3$, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Twenty to thirty day old fathead minnows were more susceptible to the toxic effects of CGA-24705 than any other life stages during the test. Significant effects on survival, growth, and reproduction were not observed among fish exposed to 0.78, 0.37, and 0.20 mg/l for 266 days. Based on significantly reduced survival of F_0 larvae after 34 days of exposure to concentrations ≥1.6 mg/l, the maximum acceptable toxicant concentration (MATC) for CGA-24705 to fathead minnow was >0.78 and <1.6 mg/l.

No quality assurance or good laboratory practice statements were included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP except for the following:

Raw water quality, analytical, and biological data were not included in the report. Raw data must be submitted for verification of the statistically-derived MATC.

Replicates A and B of the solvent control and replicate A of the dilution water control were contaminated with the test material at some point during the test (Table 3, attached).

The temperature was measured only once per week and the results of weekly temperature measurements were not reported. The solution temperature should have been continuously monitored. The actual test temperature in this test is unknown.

The age of the fathead minnow embryos used was not reported. Since hatch was complete on day 4, the eggs were probably 1-2 days old at test initiation. The SEP states that the test must be initiated with ≤24-hour old embryos.

Each test concentration and control consisted of only two replicate aquaria. Each aquarium contained two

incubation cups with 50 embryos per cup. The SEP requires the use of four replicates per concentration, with 50 embryos/replicate.

The hardness of the dilution water (21-44 mg/l as $CaCO_3$) was less than recommended (40-48 mg/l).

The light intensity used during the test was not reported.

B. **Statistical Analysis:** Raw growth data were not included in the report. Statistical analysis of the mean growth data was not attempted. The reviewer used one-way ANOVA and Dunnett's test (Toxstat version 3.3) to analyze proportional hatchability and survival data and two reproductive endpoints. Proportional data were arcsine square root transformed prior to analysis.

In several instances, 100% mortality occurred in a replicate (i.e., solvent control replicate A, 1.6 mg/l replicate B) and information on reproductive parameters could not be collected. For analysis purposes, only test levels with data from 2 replicates were included. In addition, for the 3.4 mg/l test level, F_1 survival and reproduction data were collected on embryos and larvae originally obtained from control spawners. Therefore, this level was excluded from the analysis.

The reviewer's results were similar to those of the author (see Table A1 and attached printouts 1 through 8).

C. **Discussion/Results:** This study is not scientifically sound. At some points during the exposure, both dilution water and solvent control solutions were contaminated with the test material. There was also 100% mortality in one of the solvent control replicates. The test design included only two replicates for each concentration or control. When a complete mortality occurred in one replicate, the data from the other replicate were useless since they could not be included in statistical analyses. If the test is repeated, the test design should include four replicates per concentration. Raw water quality, analytical, and biological data were not submitted for review. Based on the summarized results in the report and the reviewer's analysis of the survival and reproduction data, the MATC was >0.78 and <1.6 mg/l mean measured concentrations (geometric mean MATC = 1.1 mg/l).

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** 1) Raw water quality, analytical, and biological data were not provided for review. 2) Both control solutions were contaminated with the test material. 3) A complete mortality occurred in one of the solvent control replicates.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 03-09-93.

Table A1. Results of independent statistical analysis in mean measured concentrations (mg/l) of CGA-24705.

Parental Generation Parameter	NOEC	LOEC	Method
hatching success	3.4	---	Dunnett's
35-day survival	0.78	1.6	"
62-day survival	1.6	---	"
181-day adult survival	1.6	---	"
# eggs/female	0.78	---	"
# eggs/spawn	0.78	---	"
Progeny Generation			
hatching success	1.6	---	"
34-day survival	1.6	---	"

(---) no statistically derived LOEC available, higher test concentrations had insufficient data to be included in the analysis

Table 3 -- Measured concentrations of CGA-24705 in water during 266 days exposure of fathead minnows.

Nominal concentration (mg/l)		Mean ± S.D. (mg/l)	%RSD	ngs	# of samples
4.0	A	3.3 ± 0.5	15	2.0-4.3	31
	B	3.4 ± 0.6	18	1.4-5.0	31
	$\bar{x}$	3.4 ± 0.5		1.4-5.0	62
2.0	A	1.6 ± 0.3	19	0.74-2.3	37
	B	1.5 ± 0.4	27	0.33-2.0	36
	$\bar{x}$	1.6 ± 0.4		0.33-2.3	73
1.0	A	0.85 ± 0.26	31	0.50-2.1	35
	B	0.73 ± 0.21	29	0.16-1.2	36
	$\bar{x}$	0.78 ± 0.23		0.16-2.1	71
0.50	A	0.41 ± 0.12	29	0.021-0.56	36
	B	0.33 ± 0.11	33	0.022-0.50	37
	$\bar{x}$	0.37 ± 0.12		0.021-0.56	73
0.25	A	0.22 ± 0.06	27	0.13 -0.42	36
	B	0.17 ± 0.09	53	0.030-0.30	35
	$\bar{x}$	0.20 ± 0.08		0.030-0.42	71
Solvent control	A	-		<0.004-0.03	37
	B	-		<0.004-0.013	37
	$\bar{x}$	-		<0.004-0.03	74
control	A	-		<0.004-0.01	37
	B	-		<0.004-<0.00	36
	$\bar{x}$	-		<0.004-<0.00	73

Control contamination ?

Table 4 -- Hatching success, survival and total length of F_0 fathead minnows after 34 and 64 days exposure to CGA-24705.

Mean measured concentration (mg/l)		Hatching success (%) # live fry/100 x 100	Day 35 Survival (%) # fry/40 x 100	Day 35 Total length ± S.D. (mm)	Day 64 Survival (%) # fry/40 x 100	Day 64 Total length ± S.D. (mm)
3.4	A	89	0	-	-	-
	B	89	0	-	-	-
1.6	A	94	48[a]	21 ± 2	48	20 ± 3
	B	93	20[a]	19 ± 4	0	-
0.78	A	88	90	21 ± 4	90	30 ± 4
	B	99	85	22 ± 3	85	31 ± 5
0.37	A	91	100	20 ± 3	98	29 ± 4
	B	90	90	21 ± 3	90	30 ± 4
0.20	A	95	85	21 ± 3	80	30 ± 4
	B	88	100	21 ± 4	100	29 ± 5
solvent control	A	93	90	20 ± 4	0[b]	-
	B	89	93	21 ± 4	93	29 ± 5
control	A	85	93	19 ± 5	93	28 ± 6
	B	91	85	21 ± 3	85	30 ± 3

[a] Survival significantly (P=0.05) reduced.

[b] Fish died as a result of a suspected microbial infection between day 54 and 60.

Table 5 -- Survival and sex ratios of F_c fathead minnows exposed 181 days to CGA-24705.

DAY when thinned into spawning groups.

Mean measured concentrations (mg/l)		Survival (%) # of survivors/15 x 100	Sex ratio ♂/♀	Retained as spawners ♂/♀
3.4	A	0	-	-
	B	0	-	-
1.6	A	67	5/5	3/5
	B	0	-	-
0.78	A	100	7/6[a]	4[b]/5
	B	87	8/5	3/5
0.37	A	93	9/5	3/5
	B	100	9/6	3/6
0.20	A	100	10/5	3/5
	B	87	8/5	3/5
solvent control	A	0[c]	-	-
	B	100	13/2	4[b]/2
control	A	93	5/9	3/6
	B	87	6/7	3/6

[a] Two severely deformed fish of undetermined sex are not included in the sex ratio.

[b] An immature male fish was identified as a female at this time but later developed male characteristics.

[c] 15 fish transferred from solvent control B died between days 67 and 70 as a result of a system malfunction.

Table 6 -- Survival, growth, and sexual maturity of F_0 fathead minnows after 266 days exposure to [illegible]

Mean measured concentrations (mg/l)		Number of survivors		Number of mortalities (day 181-266)	Total length (mm)		Total weight (g)	
		male	female		male	female	male	female
3.4	A	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-
1.6	A	3	4	1	68 ± 4	53 ± 4	4.18 ± 0.75	1.33 ± 0.29
	B	-	-	-	-	-	-	-
0.78	A	4	5	0	64 ± 7	52 ± 2	3.48 ± 1.10	1.33 ± 0.16
	B	3	5	0	68 ± 3	52 ± 2	3.77 ± 0.75	1.37 ± 0.19
0.37	A	3	5	0	68 ± 1	52 ± 2	3.96 ± 0.17	1.15 ± 0.16
	B	3	6	0	70 ± 2	55 ± 3	3.86 ± 0.86	1.55 ± 0.25
0.20	A	3	5	0	67 ± 3	50 ± 4	3.49 ± 0.75	1.28 ± 0.25
	B	3	5	0	60 ± 4	51 ± 5	2.86 ± 0.72	1.17 ± 0.28
solvent control	A	-	-	-	-	-	-	-
	B	3[a]	2	0	55 ± 1	60 ± 6	3.33 ± 0.30	1.80 ± 0.59
control	A	2	6	1	63 ± 1	51 ± 3	3.42 ± 0.26	1.30 ± 0.20
	B	3	6	0	65 ± 2	53 ± 3	3.08 ± 0.44	1.33 ± 0.17

[a] One male fish removed and discarded on day 213 to adjust sex ratio.

Table 7 -- Egg production of fathead minnows chronically exposed to CGA-24705.

Mean measured concentrations (mg/l)		# females	Total spawns	Total eggs	Spawns/ female	Eggs/ female	Eggs/ spawn
3.4	A	-					
	B	-					
1.6	A	4	14	2,730	4	683	195
	B	-	-	-	-	-	-
0.78	A	5	8	740	2	148	93
	B	5	20	2,108	4	422	105
0.37	A	5	20	2,916	4	583	146
	B	6	17	3,255	3	542	191
0.20	A	5	17	3,532	3	706	208
	B	5	26	4,096	5	819	158
solvent control	A	-	-	-	-	-	-
	B	2	13	1,571	7	786	121
control	A	6	28	4,470	5	745	160
	B	6	18	2,490	3	415	138

dependent on # of female present - not analyzed

Table 8 -- Hatching success, survival and growth of F_1 fathead minnows exposed to CGA-247C5 for 34 days.

Mean measured concentrations (mg/L)		Hatching success mean ± S.D. (N)[a]	34 Days Survival (%)	34 Days Total length mean ± S.D.	34 Days Average wet weight (g)
3.4	A	92 ± 7 (3)[b]	13[c]	16 ± 1[c]	0.03[c]
	B	81 ± 7 (3)[b]	33[c]	16 ± 2[c]	0.04[c]
1.6	A	90 ± 6 (8)	90	20 ± 2	0.07
	B	91 ± 1 (3)[b]	65	21 ± 3	0.10
0.78	A	89 ± 7 (6)	80	20 ± 2	0.08
	B	90 ± 5 (9)	88	20 ± 2	0.08
0.37	A	90 ± 6 (9)	93	20 ± 2	0.08
	B	91 ± 5 (10)	90	19 ± 2	0.07
0.20	A	89 ± 7 (8)	98	21 ± 2	0.08
	B	92 ± 7 (10)	98	20 ± 2	0.07
solvent control	A	93 ± 5 (3)[b]	90	22 ± 2	0.11
	B	92 ± 3 (7)	88	22 ± 2	0.09
control	A	90 ± 7 (11)	85	22 ± 3	0.10
	B	93 ± 5 (7)	95	20 ± 2	0.06

[a] Number of egg groups used to calculate mean % hatch.

[b] Egg groups and subsequent fry transferred from control.

[c] Significantly (P=0.05) different from control.

470257-023, metolachlor, F0 hatching success
File: a:47025723.dt1 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
These two tests can not be performed because at least one group has zero variance.
Data FAIL to meet homogeneity of variance assumption.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.023	0.004	0.561
Within (Error)	7	0.048	0.007	
Total	13	0.070		

Critical F value = 3.87 (0.05,6,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.268	0.910		
2	dilution contrl	1.220	0.880	0.586	
3	0.20	1.281	0.915	-0.161	
4	0.37	1.258	0.905	0.125	
5	0.78	1.344	0.935	-0.922	
6	1.6	1.313	0.935	-0.550	
7	3.4	1.233	0.890	0.427	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

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	solvent control	2			
2	dilution contrl	2	0.171	18.8	0.030
3	0.20	2	0.171	18.8	-0.005
4	0.37	2	0.171	18.8	0.005
5	0.78	2	0.171	18.8	-0.025
6	1.6	2	0.171	18.8	-0.025
7	3.4	2	0.171	18.8	0.020

470257-023, metolachlor, F0 survival, 35 days
File: a:47025723.dt2 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.785	0.157	6.803
Within (Error)	6	0.138	0.023	
Total	11	0.924		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.276	0.915		
2	dilution contrl	1.238	0.890	0.250	
3	0.20	1.332	0.925	-0.371	
4	0.37	1.370	0.950	-0.621	
5	0.78	1.211	0.875	0.428	
6	1.6	0.615	0.340	4.354	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

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	solvent control	2			
2	dilution contrl	2	0.355	38.8	0.025
3	0.20	2	0.355	38.8	-0.010
4	0.37	2	0.355	38.8	-0.035
5	0.78	2	0.355	38.8	0.040
6	1.6	2	0.355	38.8	0.575

470257-023, metolachlor, F0 survival, 64 days
File: a:47025723.dt3 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

470257-023, metolachlor, F0 survival, 64 days
File: a:47025723.dt3 Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.457	0.291	1.610
Within (Error)	6	1.086	0.181	
Total	11	2.542		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	1.238	0.890		
2	solvent control	0.691	0.465	1.286	
3	0.20	1.299	0.900	-0.144	
4	0.37	1.339	0.940	-0.237	
5	0.78	1.211	0.875	0.063	
6	1.6	0.422	0.240	1.918	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

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	dilution contrl	2			
2	solvent control	2	0.892	100.2	0.425
3	0.20	2	0.892	100.2	-0.010
4	0.37	2	0.892	100.2	-0.050
5	0.78	2	0.892	100.2	0.015
6	1.6	2	0.892	100.2	0.650

470257-023, metolachlor, F0 survival, 181 days
File: a:47025723.dt4 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.207	0.241	1.135
Within (Error)	6	1.276	0.213	
Total	11	2.483		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	1.252	0.900		
2	solvent control	0.785	0.500	1.013	
3	0.20	1.322	0.935	-0.150	
4	0.37	1.372	0.965	-0.260	
5	0.78	1.322	0.935	-0.150	
6	1.6	0.544	0.335	1.536	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

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	dilution contrl	2			
2	solvent control	2	0.899	99.9	0.400
3	0.20	2	0.899	99.9	-0.035
4	0.37	2	0.899	99.9	-0.065
5	0.78	2	0.899	99.9	-0.035
6	1.6	2	0.899	99.9	0.565

470257-023, metolachlor, F0 reproduction, # eggs/female
File: a:47025723.dt5 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	232825.000	77608.333	3.129
Within (Error)	4	99213.000	24803.250	
Total	7	332038.000		

Critical F value = 6.59 (0.05,3,4)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	580.000	580.000		
2	0.20	762.500	762.500	-1.159	
3	0.37	562.500	562.500	0.111	
4	0.78	285.000	285.000	1.873	

Dunnett table value = 88.38 (1 Tailed Value, P=0.05, df=4,3)

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	dilution contrl	2			
2	0.20	2	13919.008	2399.8	-182.500
3	0.37	2	13919.008	2399.8	17.500
4	0.78	2	13919.008	2399.8	295.000

470257-023, metolachlor, F0 reproduction, # eggs/spawn
File: a:47025723.dt6 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	8066.375	2688.792	4.174
Within (Error)	4	2576.500	644.125	
Total	7	10642.875		

Critical F value = 6.59 (0.05,3,4)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	149.000	149.000		
2	0.20	183.000	183.000	-1.340	
3	0.37	168.500	168.500	-0.768	
4	0.78	99.000	99.000	1.970	

Dunnett table value = 88.38 (1 Tailed Value, P=0.05, df=4,3)

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	dilution contrl	2			
2	0.20	2	2243.051	1505.4	-34.000
3	0.37	2	2243.051	1505.4	-19.500
4	0.78	2	2243.051	1505.4	50.000

470257-023, metolachlor, F1 hatching success
File: a:47025723.dt7 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.003	0.001	1.183
Within (Error)	6	0.003	0.001	
Total	11	0.007		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	1.276	0.915		
2	solvent control	1.294	0.925	-0.737	
3	0.20	1.258	0.905	0.744	
4	0.37	1.258	0.905	0.778	
5	0.78	1.241	0.895	1.481	
6	1.6	1.258	0.905	0.778	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

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	dilution contrl	2			
2	solvent control	2	0.041	4.5	-0.010
3	0.20	2	0.041	4.5	0.010
4	0.37	2	0.041	4.5	0.010
5	0.78	2	0.041	4.5	0.020
6	1.6	2	0.041	4.5	0.010

470257-023, metolachlor, F1 survival, 34 days
File: a:47025723.dt8 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
These two tests can not be performed because at least one group has zero variance.
Data FAIL to meet homogeneity of variance assumption.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.130	0.026	2.186
Within (Error)	6	0.071	0.012	
Total	11	0.201		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	1.259	0.900		
2	solvent control	1.233	0.890	0.240	
3	0.20	1.429	0.980	-1.557	
4	0.37	1.276	0.915	-0.155	
5	0.78	1.162	0.840	0.891	
6	1.6	1.093	0.775	1.521	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

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	dilution contrl	2			
2	solvent control	2	0.244	27.1	0.010
3	0.20	2	0.244	27.1	-0.080
4	0.37	2	0.244	27.1	-0.015
5	0.78	2	0.244	27.1	0.060
6	1.6	2	0.244	27.1	0.125

Ecological Effects Branch One-Liner Data Entry Form

Chemical Metolachlor Shaughnessy No. 108801 Pesticide Use herbicide

AQUATIC VERTEBRATE TOX.	% AI	LC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	**% AI**	**MATC ~~LC50~~**	**DAYS**	**AFFECTED PARA.**	**STUDY/REVIEW DATES**	**MRID/ CATEGORY**	**LAB**	**RC**
1. Pimephales promelas	97.4	<0.78 mg/l* >1.6 mg/l	266	Survival	1978/1993	470257-023 INVALID	EGG	LMR
2.								
3.								

COMMENTS: a full life-cycle study * mean measured concentrations

TITLE: 470257-023, metolachlor, F0 hatching success
FILE: a:47025723.dt1
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.9300	1.3030
1	solvent control	2	0.8900	1.2327
2	dilution contrl	1	0.8500	1.1731
2	dilution contrl	2	0.9100	1.2661
3	0.20	1	0.8800	1.2171
3	0.20	2	0.9500	1.3453
4	0.37	1	0.9100	1.2661
4	0.37	2	0.9000	1.2490
5	0.78	1	0.9900	1.4706
5	0.78	2	0.8800	1.2171
6	1.6	1	0.9300	1.3030
6	1.6	2	0.9400	1.3233
7	3.4	1	0.8900	1.2327
7	3.4	2	0.8900	1.2327

470257-023, metolachlor, F0 survival, 35 days
FILE: a:47025723.dt2
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.9000	1.2490
1	solvent control	2	0.9300	1.3030
2	dilution contrl	1	0.9300	1.3030
2	dilution contrl	2	0.8500	1.1731
3	0.20	1	0.8500	1.1731
3	0.20	2	1.0000	1.4917
4	0.37	1	1.0000	1.4917
4	0.37	2	0.9000	1.2490
5	0.78	1	0.9000	1.2490
5	0.78	2	0.8500	1.1731
6	1.6	1	0.4800	0.7654
6	1.6	2	0.2000	0.4636

TITLE: 470257-023, metolachlor, F0 survival, 64 days
FILE: a:47025723.dt3
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	0.8500	1.1731
1	dilution contrl	2	0.9300	1.3030
2	solvent control	1	0.9300	1.3030
2	solvent control	2	0.0000	0.0791
3	0.20	1	0.8000	1.1071
3	0.20	2	1.0000	1.4917
4	0.37	1	0.9000	1.2490
4	0.37	2	0.9800	1.4289
5	0.78	1	0.9000	1.2490
5	0.78	2	0.8500	1.1731
6	1.6	1	0.4800	0.7654
6	1.6	2	0.0000	0.0791

TITLE: 470257-023, metolachlor, F0 survival, 181 days
FILE: a:47025723.dt4
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	0.8700	1.2019
1	dilution contrl	2	0.9300	1.3030
2	solvent control	1	1.0000	1.4413
2	solvent control	2	0.0000	0.1295
3	0.20	1	0.8700	1.2019
3	0.20	2	1.0000	1.4413
4	0.37	1	0.9300	1.3030
4	0.37	2	1.0000	1.4413
5	0.78	1	0.8700	1.2019
5	0.78	2	1.0000	1.4413
6	1.6	1	0.6700	0.9589
6	1.6	2	0.0000	0.1295

TITLE: 470257-023, metolachlor, F0 reproduction, # eggs/female
FILE: a:47025723.dt5
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	745.0000	745.0000
1	dilution contrl	2	415.0000	415.0000
2	0.20	1	706.0000	706.0000
2	0.20	2	819.0000	819.0000
3	0.37	1	583.0000	583.0000
3	0.37	2	542.0000	542.0000
4	0.78	1	148.0000	148.0000
4	0.78	2	422.0000	422.0000

TITLE: 470257-023, metolachlor, F0 reproduction, # eggs/spawn
FILE: a:47025723.dt6
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	160.0000	160.0000
1	dilution contrl	2	138.0000	138.0000
2	0.20	1	208.0000	208.0000
2	0.20	2	158.0000	158.0000
3	0.37	1	191.0000	191.0000
3	0.37	2	146.0000	146.0000
4	0.78	1	93.0000	93.0000
4	0.78	2	105.0000	105.0000

TITLE: 470257-023, metolachlor, F1 hatching success
FILE: a:47025723.dt7
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	0.9000	1.2490
1	dilution contrl	2	0.9300	1.3030
2	solvent control	1	0.9300	1.3030
2	solvent control	2	0.9200	1.2840
3	0.20	1	0.8900	1.2327
3	0.20	2	0.9200	1.2840
4	0.37	1	0.9000	1.2490
4	0.37	2	0.9100	1.2661
5	0.78	1	0.8900	1.2327
5	0.78	2	0.9000	1.2490
6	1.6	1	0.9000	1.2490
6	1.6	2	0.9100	1.2661

TITLE: 470257-023, metolachlor, F1 survival, 34 days
FILE: a:47025723.dt8
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	0.8500	1.1731
1	dilution contrl	2	0.9500	1.3453
2	solvent control	1	0.9000	1.2490
2	solvent control	2	0.8800	1.2171
3	0.20	1	0.9800	1.4289
3	0.20	2	0.9800	1.4289
4	0.37	1	0.9300	1.3030
4	0.37	2	0.9000	1.2490
5	0.78	1	0.8000	1.1071
5	0.78	2	0.8800	1.2171
6	1.6	1	0.9000	1.2490
6	1.6	2	0.6500	0.9377