7/16/92

D174857
DPBARCODE (RECORD)
036101
SHAUGHNESSY NO

REVIEW NO.

# EEB REVIEW

DATE IN: 02-26-92 OUT:
CASE # : 818802 REREG CASE #: SUBMISSION # : S412176 LIST A, B, C, D  ID # : 036101
DATE OF SUBMISSION02-12-90
DATE RECEIVED BY EFED 02-25-92
SRRD/RD REQUESTED COMPLETION DATE05-24-92
EEB ESTIMATED COMPLETION DATE05-24-92
SRRD/RD ACTION CODE/TYPE OF REVIEW 639 - Flagged Study
MRID #(S)413862-01, 413862-02
DP TYPE 001 - Submission Related Data Package
PRODUCT MANAGER, NO. W. Waldrop (71)
PRODUCT NAME(S)Trifluralin
TYPE PRODUCT FRINHD Herbicide
COMPANY NAME DowElanco
SUBMISSION PURPOSE Review fish early life stage and
INCLUDE USE(S) aquatic invertebrate life cycle
studies
COMMON CHEMICAL NAMETrifluralin

DP BARCODE: D174857

REREG CASE #

CASE: 818802 SUBMISSION: \$412176 DATA PACKAGE RECORD

BEAN SHEET

DATE: 02/24/92 Page 1 of 1

\* \* \* CASE/SUBMISSION INFORMATION \* \* \*

CASE TYPE: REREGISTRATION

ACTION: 639 FLAGGED STUDY/IMM REVIEW

CHEMICALS: 036101 Trifluralin ( a,a,a-trifluro-2,6-dinitro-N,N-dipro 100.00 %

ID#: 036101 COMPANY:

PRODUCT MANAGER: 71 WALTER WALDROP PM TEAM REVIEWER: TERRI STOWE

703-308-8062 ROOM: CS1

383

703-308-8043 ROOM: CS1

**3**05

RECEIVED DATE: 02/12/90

DUE OUT DATE: 05/13/90

#### \* \* \* DATA PACKAGE INFORMATION \* \* \*

DP BARCODE: 174857 EXPEDITE: Y DATE SENT: 02/24/92 DATE RET.: / / CHEMICAL: 036101 Trifluralin ( a,a,a-trifluro-2,6-dinitro-N,N-dipropyl-p-tol

DP TYPE: 001 Submission Related Data Package

**ADMIN DUE DATE: 05/24/92** 

LABEL: N

ASSIGNED TO DATE IN DIV : EFED

QJ 15197

BRAN: EEB

11-7/16/12

SECT: REVR : CONTR:

#### \* \* \* DATA REVIEW INSTRUCTIONS \* \* \*

Please review Trifluralin data for GLN 72-4a Fish Early Life Stage (MRID 41386202) and GLN 72-4b Life Cycle Invertebrate (Aquatic) (MRID 41386201). DowElanco is submitting this data to substitute for the GLN 72-7b `Actual Field Testing - Aquatic Organisms study. Please review as soon as possible so that the registrant can be informed as to the status of this surrogate data. Please send a copy of the review to: Terri Stowe

SRRD/RB (H7508W)

Crystal Station I - 3rd floor

#### THANK YOU!!!

For the attached reregistration case, please identify all applicable data requirements and note those for which adaquate data have not been submitted to the Agency.

\* \* \* ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION \* \* \*

DP BC BRANCH/SECTION DATE OUT DUE BACK INS

#### EEB BRANCH REVIEW

PESTICIDE NAME: Trifluralin

## 100.0 Submission Purpose:

Submission of two aquatic chronic studies:

- 1. Daphnia magna Life-Cycle (21-Day Renewal)
- 2. Rainbow Trout Early Life-Stage (Flow Through)

## 101.4 Adequacy of Toxicity Data

# A. Rainbow Trout (Early Life-Stage)

This study is scientifically sound and meets the guideline requirements for an early life-stage toxicity test using rainbow trout. Based on the most sensitive biological parameter, larvae fish length at test termination, the MATC was > 1.14 ug/L and < 2.18 ug/L mean measured concentration (geometric mean MATC = 1.58 ug/L.

## B. <u>Daphnia magna:</u>

This study is scientifically sound and meets the guideline requirements for a chronic, static renewal toxicity test using the freshwater invertebrate, <u>Daphnia magna</u>. Providing the registrant will accept the MATC value as 50.7 ppb (the highest level tested) based on mean measured concentration. There was no effect on survival, reproduction or growth. Therefore, the no-observed-effect concentration (NOEC) was 50.7 ppb based on mean measured concentration, the highest concentration tested.

Curtis E. Laird, Fishery Biologist

Ecological Effects Branch

Environmental Fate and Effects Division (H7507C)

Allen Vaughan, Acting Head-Section 2

Ecological Effects Baranch

Environmental Fate and Effects Division (H7507C)

Douglas J. Urben, Acting Chief Ecological Effects Branch

Environmental Fate and Effects Division (H7507C)

#### DATA EVALUATION RECORD

- 1. <u>CHEMICAL</u>: Trifluralin. Shaughnessey No. 036101.
- 2. <u>TEST MATERIAL</u>: Trifluralin (EL-152 compound 036352);  $\alpha, \alpha, \alpha$ -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; Lot No. 326EF8; 99.86% active ingredient.
- 3. <u>STUDY TYPE:</u> Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test. Species Tested: Daphnia magna.
- 4. <u>CITATION</u>: Grothe, D.W. and R.R. Mohr. 1990. The Chronic Toxicity of Trifluralin to *Daphnia magna* in a Static Renewal Life-Cycle Test. Laboratory Project ID C01589. Prepared by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco. EPA MRID No. 413862-01.
- 5. REVIEWED BY:

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

Date:

6. APPROVED BY:

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

Signature:

Date:

CEL. 6-29-92 AWV 7.14.92

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

signature:

Date:

7. CONCLUSIONS: This study is scientifically sound and meet the guideline requirements for a chronic, static renewal toxicity test using the freshwater invertebrate, Daphnia magna. Providing the registrant will accept the MATC value as 50.7 ppb (the highest level tested) based on mean measured concentration. There was no effect on survival, reproduction or growth. Therefore, the no-observed-effect concentration (NOEC) was  $50.7~\mu g/l$  based on mean measured concentration, the highest concentration tested.

#### DATA EVALUATION RECORD

- Trifluralin. CHEMICAL: 1. Shaughnessey No. 036101.
- TEST MATERIAL: Trifluralin (EL-152 compound 036352);  $\alpha,\alpha,\alpha$ -2. trifluoro-2,6-dinitro-N,N-dipropyl-p-to/uidine; Lot No. 326EF8; 99.86% active ingredient.
- STUDY TYPE: Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test. Species Tested: Daphnia magna.
- CITATION: Grothe, D.W. and R.R/Mohr. 1990. The Chronic Toxicity of Trifluralin to Daphnia magna in a Static Renewal Life-Cycle Test. Laboratory Project ID C01589. Prepared by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco. EPA MRID No. 41/3862-01.
- REVIEWED BY: 5.

Louis M. Rifici, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc. Signature: Silve Mi Refer 
5/23/95

## APPROVED BY:

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

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

signature: P. Kosalwat

Date: 5/22/92

Signature: Date: 7/5/42

- CONCLUSTONS: This study is scientifically sound but does 7. not meet the guideline requirements for a chronic, static renewal toxicity test using the freshwater invertebrate, Daphnia magna. Since there was no effect on survival, reproduction or growth, the maximum acceptable toxicant concentration could not be determined in this test. The noobserved-effect concentration (NOEC) was 50.7  $\mu$ g/l mean measured concentration, the highest concentration tested.
- RECOMMENDATIONS: N/A. 8.
- BACKGROUND:

- 8. <u>RECOMMENDATIONS</u>: Since this study did not establish the actual MATC or the NOEC values, the registrant can do one of the following:
  - This study is considered core providing the registrant will accept the highest level tested (50.7 ppb) as the MATC value, or
  - Conduct another study using higher dosage levels to establish the exact MATC and NOEC values.

#### 9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

#### 11. MATERIALS AND METHODS:

- A. Test Animals: First instar Daphnia magna (≤24 hours old) were obtained from in-house cultures. The adults were isolated 24 hours before the test to ensure that all neonates collected for testing were ≤24 hours old. The brood stock were held at 20 ±2°C in conditioned well water having a total hardness of 103-120 mg/l as CaCO<sub>3</sub>, a total alkalinity of 120-140 mg/l as CaCO<sub>3</sub>, and a conductivity of 230 μS/cm. The adults were fed Selenastrum capricornutum and cerophyl daily.
- glass beakers containing 200 ml of test solution. A 16-hour light/8-hour dark photoperiod was used during acclimation and testing. Light intensity was very low (<1 ft-candle) to reduce possible photodegradation of trifluralin. The test temperature was maintained at 20°C using a constant temperature water bath. The dilution water was from the same source as that used for culturing.

The test stock solution was prepared 24 hours prior to test initiation and on days 6 and 13. The test material was dissolved in acetone and stirred for 1 minute. This stock was diluted further in acetone to prepare secondary stock solutions for each test level. The test solutions were prepared by adding 300  $\mu$ l of an appropriate secondary stock to 3 l of dilution water. The solutions were stirred for 20 minutes. The final concentration of acetone in the solvent control and treatment solutions was 0.1 ml/l. The solutions were not aerated during the test.

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

## 11. MATERIALS AND METHODS:

- A. Test Animals: First instar Daphnia magna ( $\leq$ 24 hours old) were obtained from in-house cultures. The adults were isolated 24 hours before the test to ensure that all neonates collected for testing were  $\leq$ 24 hours old. The brood stock were held at 20  $\pm$ 2°C in conditioned well water having a total hardness of 103-120 mg/l as CaCO<sub>3</sub>, a total alkalinity of 120-140 mg/l as CaCO<sub>3</sub>, and a conductivity of 230  $\mu$ S/cm. The adults were fed Selenastrum capricornutum and cerophyl daily.
- B. Test System: The test chambers were covered 250-ml glass beakers containing 200 ml of test solution. A 16-hour light/8-hour dark photoperiod was used during acclimation and testing. Light intensity was very low (<1 ft-candle) to reduce possible photodegradation of trifluralin. The test temperature was maintained at 20°C using a constant temperature water bath. The dilution water was from the same source as that used for culturing.

The test stock solution was prepared 24 hours prior to test initiation and on days 6 and 13. The test material was dissolved in acetone and stirred for 1 minute. This stock was diluted further in acetone to prepare secondary stock solutions for each test level. The test solutions were prepared by adding 300  $\mu$ l of an appropriate secondary stock to 3 l of dilution water. The solutions were stirred for 20 minutes. The final concentration of acetone in the solvent control and treatment solutions was 0.1 ml/l. The solutions were not aerated during the test.

- C. <u>Dosage</u>: Twenty-one-day, static-renewal, life-cycle chronic toxicity test. Six nominal concentrations (2.25, 4.5, 9.0, 18.0, 36.0, and 72.0 μg/l), an acetone control (0.1 ml/l), and a dilution water control were selected for the test.
- D. <u>Design</u>: Ten replicates containing one daphnid each were used for each concentration. Distribution of the animals to the test chambers followed a stratified random assignment. The test solutions were renewed on days 0, 2, 3, 5, 7, 9, 10, 12, 14, 16, 17, and 19. The daphnids were fed Selenastrum capricornutum at a density of 150,000 cells/ml during the first 7 days and

300,000 cells/ml during the last 14 days of the test. Cerophyl was added at a concentration of 2 ml/l.

Survival was monitored daily. Reproduction (number of neonates produced) and behavioral effects were determined at each renewal and at test termination. Behavior was not monitored on days 10 and 17. Observations were made of the presence of eggs in the brood chamber, ephippia, expelled eggs, and the production of males. The number of days until first brood release was also noted. The body length of the adult daphnids was measured to the nearest 0.01 mm using a Whipple eye-piece micrometer fitted on a compound microscope.

The dissolved oxygen concentration (DO), pH, and temperature were measured in each test vessel at test initiation and termination. At each renewal, the above parameters were measured in one replicate of each test level before and after renewal. The temperature of the water bath was monitored continuously. The hardness, alkalinity, and conductivity of the dilution water and highest test level were determined at initiation, and on test days 7 and 14. Total ammonia was determined in the acetone control and the highest test level at initiation and termination.

The concentration of trifluralin in the freshly prepared solutions was determined at test initiation and on days 7 and 14. On days 5, 12, and 21, composite samples were taken of the old test solutions for analysis. The analytical method used was gas chromatography.

- E. <u>Statistics</u>: The responses in the treatment concentrations were compared to those of the solvent control daphnids. The reproduction (number of neonates per female) and length data were analyzed using a one-tailed Dunnett's test. Neonates produced from females which died during the study were excluded from the statistical analysis.
- 12. REPORTED RESULTS: Preliminary work with trifluralin indicated that the compound would be stable under the conditions used in the test. However, "The concentrations of trifluralin in the exposure solutions declined during each 48-hour renewal period." Measured concentrations in the freshly-prepared and old solutions are presented in Table 2 (attached). The reported mean measured concentrations were 1.57, 3.19, 6.53, 13.7, 26.2, and 50.7

 $\mu$ g/l. These values represented 70-76% of nominal concentrations. Trifluralin was detected in the dilution water control at test termination.

No treatment-related physical signs of toxicity were observed during the test. One daphnid was accidentally killed on day 19 in the dilution water control and one daphnid was lost from the 6.53  $\mu$ g/l exposure on day 12. Temporary hypoactivity was observed in at least one daphnid from the 6.53, 13.7, and 50.7  $\mu$ g/l exposures. Ten percent immobilization was observed at 3.19, 13.7, and 26.2  $\mu$ g/l and 20% immobilization occurred at 50.7  $\mu$ g/l. "This immobilization does not appear to be related to increasing concentrations of trifluralin."

Reproduction was significantly decreased at 13.7  $\mu$ g/l only. "This decrease was not part of a concentration-related response." Release of the first brood occurred on days 9 and 10 for all test levels (Table 3, attached). "No ephippia or males were observed at any treatment or control replicate during the study." Daphnid length was not affected by exposure to trifluralin (Table 4, attached).

The mean water quality for each concentration and the controls during the 21-day exposure are presented in Table 1 (attached). The DO in the freshly prepared solutions ranged from 8.1 to 9.6 mg/l and in the old solutions from 4.2 to 8.6 mg/l. Increased algal respiration due to the low light conditions used during the test and the presence of neonates were cited as causes for the low DO observed between renewals. The hardness, alkalinity, and conductivity of the test solutions were 120-137 mg/l as CaCO<sub>3</sub>, 145-155 mg/l as CaCO<sub>3</sub>, and 232-291  $\mu$ S/cm, respectively. The concentration of un-ionized ammonia was <0.001 mg/l.

13. <u>STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:</u>
The chronic no-observed-effect concentration of trifluralin for Daphnia magna was 50.7 μg/l.

Quality Assurance and Good Laboratory Practice Statements were included in the report indicating adherence to USEPA GLP Regulations (40 CFR Part 160). The dates and types of quality assurance inspections were also reported.

- 14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:
  - A. <u>Test Procedure</u>: The test procedures were generally in accordance with the SEP and ASTM (1988), except for the following:

The physical characteristics of the test material (i.e., physical state, appearance) was not reported.

Fifteen to 30 minute transition periods between light and dark are recommended by ASTM. Transition periods were not used in this study.

The acclimation of the broodstock was not fully described. The broodstock must be acclimated to the test conditions for at least two generations or 21 days.

The light intensity used (<1 ft-candle) was lower than recommended (400-800 lux).

The test design used (10 replicates containing one daphnid each) differed from that recommended in the SEP (7 replicates containing one daphnid each and 3 replicates containing 5 daphnids each).

- B. Statistical Analysis: The total young produced per female was analyzed using one-way analysis of variance (ANOVA) and Bonferroni's t-test (Toxstat 3.3). Adult daphnid length data failed Bartlett's test for homogeneity of variance and was analyzed using the Wilcoxon Rank-Sum test. Survival was analyzed using Fisher's Exact tests. The results were similar to those of the authors (see attached printouts 1 through 7). The authors determined that reproduction at 13.7  $\mu$ g/l was significantly affected. The reviewer's comparison of reproduction at 13.7  $\mu$ g/l to that of the solvent control indicated no significant difference (see attached printout 3).
- C. <u>Discussion/Results</u>: This study is scientifically sound but does not meet the guideline requirements for a chronic, static renewal toxicity test using the freshwater invertebrate, *Daphnia magna*. The effects observed during the study (i.e., hypoactivity and immobilization) were not significantly increased in the exposures compared to the control and did not appear to follow a clear concentration-response. Since there was no effect on survival, reproduction or growth, the maximum-acceptable-toxicant concentration could not be determined in this test. The no-observed-effect concentration (NOEC) was 50.7 μg/l mean measured concentration, the highest concentration tested.
- D. Adequacy of the Study:

C. Discussion/Results: This study is scientifically sound but does not meet the guideline requirements for a chronic, static renewal toxicity test using the freshwater invertebrate, Daphnia magna. The effects observed during the study (i.e., hypoactivity and immobilization) were not significantly increased in the exposures compared to the control and did not appear to follow a clear concentration-response. Since there was no effect on survival, reproduction or growth, the maximum-acceptable-toxicant concentration could not be determined in this test. The no-observed-effect concentration (NOEC) was  $50.7~\mu g/l$  mean measured concentration, the highest concentration tested.

## D. Adequacy of the Study:

- (1) Classification: Core providing the registrant will accept the MATC value as 50.7 ppb).
- (2) Rationale: Since there was no effect on survival, reproduction or growth, the maximum acceptable toxicant concentration was greater than the highest level tsted (50.7 ppb)
- (3) Repairability: N/A providing the registrant accept the MATC value as 50.7 ppb).
- 15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 04-28-92.

REFERENCES: ASTM. 1988. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with Daphnia magna. E 1193-87.

ages	through \( \sum_{\text{are not included.}} \)
<u> </u>	
	material not included contains the following type of mation:
	Identity of product inert ingredients.
•	Identity of product impurities.
<u>.</u>	Description of the product manufacturing process.
• • •	Description of quality control procedures.
•	Identity of the source of product ingredients.
·	Sales or other commercial/financial information.
	A draft product label.
	The product confidential statement of formula.
_/	Information about a pending registration action.
<u> </u>	FIFRA registration data.
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he v o	information not included is generally considered confidentia coduct registrants. If you have any questions, please contact

TITLE: 413862-01, Trifluralin, total young per female a:41386201.dt1

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	acetone control	1	140.0000	140.0000	
1	acetone control	2	109.0000	109.0000	
1	acetone control	3	100.0000	100.0000	
1	acetone control	4	75.0000	75.0000	
1	acetone control	5	47.0000	47.0000	, ž
1	acetone control	6	97.0000	97.0000	
1	acetone control	7	91.0000	91.0000	
-1	acetone control	8	141.0000	141.0000	
ī	acetone control	9	135.0000	135.0000	
1	acetone control	10	110.0000	110.0000	
2	dilut. control	1	114.0000	114.0000	
2	dilut. control	2	144.0000	144.0000	•
2	dilut. control	3	51.0000	51.0000	
2	dilut. control	4	131.0000	131.0000	
2	dilut. control	5	107.0000	107.0000	
2	dilut. control	6	141.0000	141.0000	
2	dilut. control	7	102.0000	102.0000	
2	dilut. control	8	132.0000	132.0000	
2	dilut. control	9	162.0000	162.0000	
ร	1.57	ī	94.0000	94.0000	
3 3	1.57	2	119.0000	119.0000	
3	1.57	3	61.0000	61.0000	s
3	1.57	4	80.0000	80.0000	
. 3	1.57	5	114.0000	114.0000	
3	1.57	6	126.0000	126.0000	
3	1.57	7	71.0000	71.0000	
3	1.57	8	114.0000	114.0000	
, 3	1.57	9	89.0000	89.0000	
3	1.57	10	106.0000	106.0000	
4	3.19	1	133.0000	133.0000	
4	3.19	2	65.0000	65.0000	*
4	3.19	3	113.0000	113.0000	
	3.19	4	78.0000	78.0000	
4	3.19	5	96.0000	96.0000	
4 4	3.19	6	138.0000	138.0000	
	3.19	7	64.0000	64.0000	
4		8	82.0000	82.0000	
4	3.19 3.19	9	150.0000	150.0000	
4	3.19	9	150.0000	150.0000	

TITLE: 413862-01, Trifluralin, total young per female

FILE: a:41386201.dt1

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

	TRANS VALUE	VALUE	REP	IDENTIFICATION	GRP
	70.0000	70.0000	1	6.53	5
	120.0000	120.0000	2	6.53	5 5 5
	53.0000	53.0000	3	6.53	5
	109.0000	109.0000	4	6.53	<b>∞</b> 5
	36.0000	36.0000	5	6.53	5
	96.0000	96.0000	6	6.53	5
	122.0000	122.0000	7	6.53	5 5 5
	33.0000	33.0000	8	6.53	5
	127.0000	127.0000	. 9	6.53	5
	95.0000	95.0000	1	13.7	6
•	100.0000	100.0000	2	13.7	6
	26.0000	26.0000	.3	13.7	6
	116.0000	116.0000	4	13.7	6
	29.0000	29.0000	5	13.7	6
	53.0000	53.0000	6	13.7	6
	79.0000	79.0000	7	13.7	6
	51.0000	51.0000	8	13.7	6
	89.0000	89.0000	9	13.7	6
	105.0000	105.0000	1	26.2	7
	103.0000	103.0000	2	26.2	7
	131.0000	131.0000	3	26.2	7
	123.0000	123.0000	4	26.2	7
	128.0000	128.0000	5	26.2	7
	111.0000	111.0000	6	26.2	7
,	112.0000	112.0000	7	26.2	7
	102.0000	102.0000	8	26.2	7
	118.0000	118.0000	9	26.2	7
	163.0000	163.0000	1	50.7	.8
	122.0000	122.0000	2	50.7	8
	130.0000	130.0000	3	50.7	8
	172.0000	172.0000	4	50.7	8
	99.0000	99.0000	5	50.7	8
	81.0000	81.0000	.5 6	50.7	
	144.0000	144.0000	7	50.7	8
e N	161.0000	161.0000	<b>8</b> ,	50.7 50.7	8

Chi-square test for normality: actual and expected frequencies Data PASS normality test. Continue analysis.

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

# 413862-01, Trifluralin, total young per female

413862-01, Trifluralin, total young per female							
t-test of Solvent and	d Blank Control	s Ho:GRP1 ME	AN = GRP2 MEAN				
GRP1 (SOLVENT CRTL) MEAN = GRP2 (BLANK CRTL) MEAN = DIFFERENCE IN MEANS =	120.4444	CALCULATED t VALUE = DEGREES OF FREEDOM =	-1.1192 17				
TABLE t VALUE (0.05 (2),17) TABLE t VALUE (0.01 (2),17)	= 2.110 NO = 2.898 NO	significant different significant differen	ce at alpha=0.05 ce at alpha=0.01				
	ANOVA TABL	E					
SOURCE DF	SS	MS	F				
Between 7 Within (Error) 65 Total 72	24176.683 57161.344	3453.812 879.405	3.927				
Critical F value = 2.17 Since F > Critical F RE BONFERRONI T-TEST	JECT Ho:All gr	oups equal 2 Ho:Cont	rol <treatment< td=""></treatment<>				
GROUP IDENTIFICATION	TRANSFORMED	MEAN CALCULATED IN					
1 acetone control 2 dilut. control 3 1.57 4 3.19 5 6.53 6 13.7 7 26.2 8 50.7	104.500 120.444 97.400 102.111 85.111 70.889 114.778	104.500 120.444 97.400 102.111 85.111 70.889	0.175 1.423 2.467 -0.754				
Bonferroni T table value =							
BONFERRONI T-TEST	- TABLE 2 OF		rol <treatment< td=""></treatment<>				
GROUP IDENTIFICATION	REPS (IN C	num Sig Diff % of ORIG. UNITS) CONTROL	DIFFERENCE FROM CONTROL				
1 acetone control 2 dilut. control 3 1.57 4 3.19 5 6.53 6 13.7 7 26.2 8 50.7	10 9 10 9 9 9 9	34.391 32.9 33.473 32.0 34.391 32.9 34.391 32.9 34.391 32.9 34.391 32.9 35.504 34.0	7.100 2.389 19.389 33.611				

# PRINTOUT #

413862-01, Trifluralin, daphnid length at termination a:41386201.dt2 TITLE:

FILE:

NUMBER OF GROUPS: 8 TRANSFORM: NO TRANSFORMATION

GRP	IDENTIF	ICATION	REP	VALUE	TRANS VALUE	
1	acetone	control	1	4.5400	4.5400	
i	acetone		2	4.6600	4.6600	
1	acetone		3	4.1400	4.1400	
ī	acetone		4	4.5800	4.5800	•
ī	acetone		5	4.3600	4.3600	
ī	acetone		5 6	4.3000	4.3000	
ī	acetone		7	4.3000	4.3000	
1	acetone		8	4.3200	4.3200	
ī	acetone		, 9	4.1400	4.1400	•
î	acetone		10	4.2600	4.2600	
2	dilut.		ī	4.5000	4.5000	
2	dilut.		2	4.6600	4.6600	
2	dilut.	. 1.	3	4.6600	4.6600	
2	dilut.		4	4.5800	4.5800	3. *** *** *** *** *** *** *** *** *** *
2	dilut.		5	4.5000	4.5000	
2	dilut.		6	4.5800	4.5800	
2	dilut.		7	4.3000	4.3000	
2	dilut.		8	4.3200	4.3200	
2	dilut.		9	4.1400	4.1400	
3		1.57	1	4.3200	4.3200	
- 3	*	1.57	2	4.1200	4.1200	
3		1.57	3	4.4400	4.4400	
3		1.57	4	4.2600	4.2600	
3		1.57	5	4.3000	4.3000	
3		1.57	6	4.5800	4.5800	
. 3		1.57	7	4.4400	4.4400	
3		1.57	8	4.1200	4.1200	
3		1.57	9	4.5000	4.5000	
3		1.57	10	4.5000	4.5000	
4		3.19	1	4.6200	4.6200	
4		3.19	2	4.4400	4.4400	
4		3.19	3	4.4000	4.4000	
4		3.19	4	4.3000	4.3000	
4		3.19	5	4.5000	4.5000	ē.
4		3.19	6	4.5800	4.5800	
4		3.19	7	4.5400	4.5400	
4		3.19	8	4.3200	4.3200	
4	and the second second	3.19	9	4.7600	4.7600	

TITLE: 413862-01, Trifluralin, daphnid length at termination

FILE: a:41386201.dt2 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8 6.53 4.4400 4.4400 5 2 4.4400 6.53 4.4400 4.5000 5 3 4.5000 6.53 5 4.3600 6.53 4.3600 4.5800 5 6.53 4.5800 4.5000 5 6.53 6 4.5000 5 6.53 7 4.4800 4.4800 5 4.6200 6.53 4.6200 5 4.5400 6.53 9 4.5400 4.5400 6 4.5400 13.7 1 4.5400 6 2 4.5400 13.7 4.6200 6 13.7 3 4.6200 6 13.7 4 4.4400 4.4400 4.4800 4.4800 6 13.7 5 6 6 4.6200 4.6200 13.7 4.5800 6 13.7 7 4.5800 4.3600 4.3600 6 13.7 8 6 13.7 9 4.3200 4.3200 7 1 4.4000 4.4000 26.2 4.4800 4.4800 7 26.2 2 4.4800 7 26.2 3 4.4800 7 4.6200 4.6200 26.2 4 4.5000 4.5000 7 5 26.2 7 4.5000 4.5000 26.2 7 7 4.5400 4.5400 26.2 4.5000 7 8 4.5000 26.2 9 4.2200 4.2200 7 26.2 8 50.7 1 4.7200 4.7200 4.5000 4.5000 8 50.7 4.5800 4.5800 8 3 50.7 4.3600 4.3600 8 50.7 4.6200 4.6200 50.7 5 8 3.7200 8 50.7 6 3.7200

Chi-square test for normality: actual and expected frequencies Data PASS normality test. Continue analysis.

7

50.7

50.7

8

Bartletts test for homogeneity of variance Data FAIL homogeneity test at 0.01 level. Try another transformation.

4.6600

4.6600

4.6600

413862-01, Trifluralin, daphnid length at termination File: a:41386201.dt2 Transform: NO TRANSFORMATION

t-test of Solvent and	Blank Controls	<b>5</b>	Ho:GRP1 N	MEAN = G	RP2 MEAN
GRP1 (SOLVENT CRTL) MEAN = GRP2 (BLANK CRTL) MEAN = DIFFERENCE IN MEANS =	4.4711	CALCULATED DEGREES OF	t VALUE FREEDOM	= -1 = 17	.3507
TABLE t VALUE (0.05 (2),17) = TABLE t VALUE (0.01 (2),17) = WILCOXON RANK SUM TEST W/ BOX	= 2.898 NO	significan significan	t differe	ence at	alpha=0.01
GROUP IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	sig
	4.471 4.358 4.496 4.496 4.500 4.471	103.50 110.50 111.50 112.50	60.00 60.00 60.00	9 10 9 9 9	

Critical values use k = 7, are 1 tailed, and alpha = 0.05

# SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICAT	ION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)	· ·
1 2 3 4 5		CONTROL control 1.57 3.19 6.53 13.7 26.2	10 10 10 10 10 10	0 0 0 1 0		
7		50.7	10	2		_

Shaughnessey #	036161	Chemical Name Treffeets live	Chemical Class	Раве	of /
Study/Species/Lab/ MRID #	Chemical % a.i.	Results		Reviewer/ Date	Validation Status
Chronic Fish		Concentrations Tested (pp) =			
Species:		MATC - > pp			
Lab:		Effected Parameters -			
MRID #		Control Mortality (%) - Solvent Comments:	Solvent Control Mortality (%) -		
Chronic Invertebrate	49.56	Concentrations Tested (pp $\frac{1}{b}$ ) - $\frac{151}{100}$	57, 2,19, 6,53,12,12,136.7		
Species: Duplinia magni	- may my	MATC -> 50.7 < pp pp p		THIC	Suptemental

Chronic Invertebrate $99.50$	Concentrations Tested (pp b) = $157, 319, (.63, 18.7, 20.7)$	
Species: Duphnin mayne	$\frac{1}{\text{MATC}} = \frac{50.7}{\text{A}} < \frac{\text{ppb}}{\text{pb}}.$	LAIR Super
Lab: Lilly Research Laboratorns	Effected Parameters - 110 MC.	118717
	Control Mortelity (2) = (2) Solvent Control Mortality (2) = (	

MRID # 413562-01

Comments:
\* White Maistled to went but her?

# DATA EVALUATION RECORD

- CHEMICAL: Trifluralin. 1. Shaughnessey No. 036101.
- TEST MATERIAL: Trifluralin (compound 036352);  $\alpha, \alpha, \alpha$ -2. trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; Lot No. 326EF8; 99.86% active ingredient.
- STUDY TYPE: Freshwater Fish, Early Life-Stage Flow-Through 3. Toxicity Test. Species Tested: Rainbow Trout (Salmo gairdneri).
- CITATION: Adams, E.R., P.J. Cocke, and M.D. Gunnoe. The Toxicity of Trifluralin to Rainbow Trout (Salmo gairdneri) in a 48-Day Early Life-Stage Study. Laboratory Project ID: F02489. Prepared by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco. MRID No. 413862-02.

#### 5. REVIEWED BY:

Louis M. Rifici, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc. Signature: Jours m. Refeer
5122/97

Date:

#### APPROVED BY: 6.

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

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

signature: P. Kosalwat

Date: 5/22/92

signature: Henry T Craw

Date:

- **CONCLUSIONS:** This study is scientifically sound and meets 7. the guideline requirements for an early life-stage toxicity test using rainbow trout. Based on the most sensitive biological parameter, larval fish length at test termination, the MATC was >1.14  $\mu$ g/l and <2.18  $\mu$ g/l mean measured concentrations (geometric mean MATC = 1.58  $\mu$ g/l).
- RECOMMENDATIONS: N/A. 8.

## 9. BACKGROUND:

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

#### 11. MATERIALS AND METHODS:

- A. Test Animals: Rainbow trout (Salmo gairdneri) embryos (early eyed-stage) were obtained from a commercial supplier in McMillan, WA. The embryos were held in test dilution water in an incubator for 2 days prior to testing. The water had a hardness of 120 mg/l as  $CaCO_3$ , an alkalinity of 125 mg/l as  $CaCO_3$ , a conductivity of 220  $\mu$ S/cm, and an average temperature of 12.8°C. "Only embryos visually inspected and judged to be in good condition were used in this study."
- B. Test System: A proportional diluter delivered test solution or control water to each of four replicate test chambers per concentration. A stock solution of the test material was prepared in acetone. The diluter stock solution, a nominal concentration of 78.5 μg/l, was prepared daily using 20 ml of the acetone stock and: 205 l of dilution water in a stainless steel barrel. An automated injector delivered acetone to the solvent control solution to provide a concentration of 0.1 ml acetone/l. After day 1 until the end of the test, the concentration of acetone in the solvent control was reduced to 0.02 ml/l which was equivalent to the concentration found in the highest exposure solution.

The eggs were housed in glass egg cups. Each cup was placed inside a vessel equipped with a self starting siphon designed to fill and partially empty with each diluter cycle. The larvae were exposed in 10-gallon glass aquaria containing approximately 18 l of test solution (solution depth of 15 cm). The test solution overflowed from each aquarium through a hole drilled in the side. The test aquaria were arranged in a randomized block design on the test rack.

The dilution water was well water obtained on-site. The water was treated to remove iron, 50% of the mineral content (using electrodialysis), and excess CO<sub>2</sub> (to adjust pH). The water was stored in underground tanks and warmed or cooled to test temperature before delivery to the diluter system.

The laboratory was maintained on a 16-hour daylight photoperiod during acclimation and testing. The light intensity was below 22  $\mu E/m^2/sec$ .

- C. <u>Dosage</u>: Forty-eight-day flow-through test (30 days post-swim up). Five nominal concentrations (0.95, 1.9, 3.8, 7.6, and 15.2  $\mu$ g/l), a dilution water control, and a solvent control (0.02-0.1 ml acetone/l) were used.
- D. <u>Design</u>: Twenty embryos were selected and placed in each incubation cup/exposure chamber. Four replicates were used per concentration. The biomass of fish at test termination did not exceed 0.1 g/l/day or 0.5 g/l at any time.

Egg survival and mortality were recorded daily and dead eggs were carefully removed from the cups. When hatching was complete, the number of dead embryos, live or dead larvae, and deformed larvae were recorded. The surviving larvae were then transferred to the test aquaria. The aquaria were observed daily for dead and deformed larvae and any dead larvae were removed. Behavioral effects were noted. The aquaria were cleaned of excess food and fecal material at least 3 times per week.

The trout were fed salmon starter meal 2-3 times daily throughout the test.

At test termination, the total length of larvae from each replicate was determined photographically. The larvae were also weighed (blotted dry) to the nearest 0.0001 g.

The temperature, dissolved oxygen concentration, and pH were measured daily in one replicate of the controls and each treatment level. The temperature was also measured continuously in one control aquarium with a 7-day temperature recorder. The hardness, alkalinity, total ammonia, and conductivity were measured in one replicate of the solvent control and highest test level at test initiation and at least weekly thereafter.

Concentrations of Trifluralin were measured on study days 0, 7, 14, 21, 28, 33, 42, and 48. The samples were collected from the four replicates of each concentration and pooled before analysis by gas chromatography.

E. <u>Statistics</u>: Time to hatch, hatchability, larval survival at hatch, and larval survival at termination data were tabulated for each replicate. Survival at hatch and at termination were arcsine square root

transformed then analyzed using Dunnett's test. A weighted analysis of variance (ANOVA) procedure was used to analyze the length and weight data. Larvae that were inadvertently killed during tank cleaning were excluded from the analyses. All hypothesis testing was performed at  $\alpha$ =0.05.

12. REPORTED RESULTS: The mean measured concentrations were 0.59, 1.14, 2.18, 4.32, and 8.81  $\mu$ g/l. These values represented 57 to 62% of nominal concentrations (Table 1, attached). No trifluralin was detected in the controls.

The time to hatch, hatchability of the eggs, and larval survival at hatch in the treatment replicates were not significantly different from the controls (Table 3, attached). At test termination, the survival of larval rainbow trout at  $8.81~\mu g/l$  was significantly reduced compared to the pooled controls (Table 4, attached).

No treatment-related behavioral effects were observed in larvae exposed to test concentrations  $\leq 4.32~\mu g/l$  and no treatment-related larval deformities were observed. The growth of the dilution water control larvae and solvent control larvae were not significantly different so the two groups were pooled prior to analysis. Total length at concentrations  $\geq 2.18~\mu g/l$  was significantly lower than that of the pooled control (Table 4, attached). Body weight was significantly reduced at trifluralin concentrations  $\geq 4.32~\mu g/l$  compared the pooled controls (Table 4, attached).

The summarized water quality data are presented in Table 2 (attached). The temperature of the test solutions ranged from 12.3 to 13.2°C. Dissolved oxygen ranged from 5.8 to 11.0 mg/l. A diluter malfunction resulted in DO levels of 5.8 and 6.4 mg/l in the two highest test levels on day 24. The malfunction was repaired and all solutions were normal by day 25. The pH values ranged from 7.4 to 8.5. The conductivity, hardness, and alkalinity in the solvent control and 8.81  $\mu$ g/l test solutions averaged 196  $\mu$ S/cm, 128 mg/l as CaCO3, and 153 mg/l as CaCO3, respectively.

13. <u>STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:</u>
The chronic no-observed-effect concentration of trifluralin for rainbow trout was 1.14  $\mu$ g/l.

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in compliance with EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance inspections were also reported.

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

A. <u>Test Procedure</u>: The test procedures were generally in accordance with the SEP and ASTM (1987), except for the following:

The physical characteristics of the test material (i.e., physical state, appearance) was not described.

This test used eyed-stage trout embryos which hatched 7-11 days after test initiation. The SEP states if eyed-stage eggs are used, they must not hatch until at least 10 days after test initiation. However, ASTM states that the embryos must not hatch until at least 7 days after test initiation.

The number of days to swim-up was not reported.

The fish were fed salmon starter 2-3 times daily. ASTM recommends feeding 4 times daily (2 times daily on weekends).

A 30-minute transition period between light and dark is recommended in the SEP. A transition period was not used in the study.

The number of volume replacements used in the test was not reported.

The accuracy of the flow-splitting mechanism used to deliver the test solution to the replicate aquaria should be checked frequently during the exposure. The report does not state if the accuracy of the mechanism was checked.

The report did not indicate whether food was withheld from the fish during the last 24 hours of the test. The SEP recommends discontinuing feeding at least 24 hours prior to test termination.

B. Statistical Analysis: The number of days to hatch in the treatment levels were compared to the solvent control using one-way ANOVA and Dunnett's test (Toxstat Version 3.3). Larval survival data at hatch were not normally distributed and therefore were analyzed using Steel's Many-One Rank test. Larval survival at test termination was analyzed using one-way ANOVA and Dunnett's test. Survival in the treatments were compared to survival in the solvent control for both analyses. The results were the same as those as of the

authors' (see attached printouts 1 through 5). The reviewer used two-way ANOVA and Bonferroni's test (Crunch Version 3) to analyze the length and wet weight data. The length and weight of fish exposed to trifluralin concentrations  $\geq 4.32~\mu g/l$  were significantly lower than those of the solvent control and dilution water control fish (see attached printouts 6 through 9). These results are similar to the authors, but the authors' no observed effect concentration (NOEC) for length (1.14  $\mu g/l$  mean measured concentration) was more conservative than the reviewer's. There was no significant difference between the growth of the dilution water control fish and solvent control fish.

Discussion/Results: This study is scientifically sound and meets the guideline requirements for an early life-stage toxicity test using rainbow trout. Based on the most sensitive biological parameter, larval fish length at test termination, the maximum acceptable toxicant concentration (MATC) was >1.14  $\mu$ g/l and <2.18  $\mu$ g/l mean measured concentrations (geometric mean MATC = 1.58  $\mu$ g/l).

# D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.
- 15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 04-27-92.

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	Identity of product inert ingredients.
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TITLE: 413862-02, trifluralin, days to hatch

FILE: a:41386202.dt1

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	-
1	acetone control	1	10.0000	10.0000	*
1	acetone control	2	10.0000	10.0000	4
1	acetone control	3	8.0000	8.0000	
1	acetone control	4	8.0000	8.0000	
2	control	1	8.0000	8.0000	w.
2	control	. 2	11.0000	11.0000	
2	control	2 3	8.0000	8.0000	.4
2	control	4	10.0000	10.0000	
3	0.59	. 1	9.0000	9.0000	
3	0.59	2	11.0000	11.0000	
	0.59	3	10.0000	10.0000	
3 3	0.59	4	10.0000	10.0000	
4	1.14	1	8.0000	8.0000	
4	1.14	2	9.0000	9.0000	•
4	1.14	3	8.0000	8.0000	
4	1.14	4	9.0000	9.0000	
5	2.18	1	8.0000	8.0000	
5	2.18	2	11.0000	11.0000	, t
5	2.18	3	8.0000	8.0000	
5	2.18	4	9.0000	9.0000	
6	4.32	1	8.0000	8.0000	
6	4.32	2	9.0000	9.0000	
6	4.32	, 3	11.0000	11.0000	
6	4.32	4	8.0000	8.0000	
7	8.81	i	8.0000	8.0000	
7	8.81		8.0000	8.0000	
7	8.81	2	8.0000	8.0000	
7	8.81	4	7.0000	7.0000	•

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 Within (Error)	6 21	11.357 26.500	1.893 1.262	1.500
Total	27	37.857		

Critical F value = 2.57 (0.05,6,21) Since F < Critical F FAIL TO REJECT Ho: All groups equal

413862-02, trifluralin, days to hatch File: a:41386202.dt1 Transform: NO TRANSFORMATION

4	DUNNETTS TEST - T	Ho:Control <treatment< th=""></treatment<>			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1 2 3 4 5 6 7	acetone control control 0.59 1.14 2.18 4.32 8.81	9.000 9.250 10.000 8.500 9.000 9.000 7.750	9.000 9.250 10.000 8.500 9.000 9.000 7.750	-0.315 -1.259 0.629 0.000 0.000	

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

	DUNNETTS TEST - T	ABLE 2 OF	2 Ho:Control <treatment< th=""></treatment<>			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL	
1	acetone control	4				
2	control	4	1.954	21.7	-0.250	
3	0.59	4	1.954	21.7	-1.000	
. A	1.14	4	1.954	21.7	0.500	
5	2.18	4	1.954	21.7	0.000	
6	4.32	4	1.954	21.7	0.000	
7	8.81	4	1.954	21.7	1.250	

TITLE: 413862-02, trifluralin, larval survival at comp, hatch

FILE: 41386202.dt2

TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	acetone control	1	1.0000	1.4588	
1 *	acetone control	2	1.0000	1.4588	
1	acetone control	3	1.0000	1.4588	
1	acetone control	4	1.0000	1.4588	
2	control	1	0.9500	1.3453	
2	control	2	0.9500	1.3453	
2	control	3	1.0000	1.4588	
2	control	4	1.0000	1.4588	•
3	0.59	i	1.0000	1.4588	
3	0.59	2	0.9500	1.3453	
3	0.59	3	0.9500	1.3453	
3	0.59	4	1.0000	1.4588	
4	1.14	1	1.0000	1.4588	
4	1.14	2	1.0000	1.4588	
4	1.14	3	0.9500	1.3453	
4	1.14	4	1.0000	1.4588	
, <u> </u>	2.18	1	1.0000	1.4588	,
5	2.18	2	0.9500	1.3453	ų.
5	2.18	3	1.0000	1.4588	
5	2.18	4	0.9500	1.3453	
6	4.32	1	0.9500	1.3453	
6	4.32	2	1.0000	1.4588	1
6	4.32	3	0.9500	1.3453	
6	4.32	3 4	1.0000	1.4588	
7	8.81	1	0.9500	1.3453	
7	8.81	2	1.0000	1.4588	
7	8.81	3	0.9500	1.3453	
7	8.81	4	1.0000	1.4588	

Shapiro Wilks test for normality Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
 1	acetone control	1.459				
2	control	1.402	14.00	10.00	4.00	
3	0.59	1.402	14.00	10.00	4.00	
4	1.14	1.430	16.00	10.00	4.00	
5	2.18	1.402	14.00	10.00	4.00	
6	4.32	1.402	14.00	10.00	4.00	
7	8.81	1.402	14.00	10.00	4.00	

TITLE: 413862-02, trifluralin, larval survival at termination

FILE: a:41386202.dt3

TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	acetone control	1	0.7220	1.0154	
1	acetone control		0.9500	1.3453	
1	acetone control		0.7220	1.0154	
1	acetone control	4	0.8950	1.2408	
2	control	1	0.8950	1.2408	
2	control		0.7890	1.0935	
2	control		0.7890	1.0935	
2	control		0.9000	1.2490	
3	0.59	1	0.9000	1.2490	
3	0.59	2	0.8950	1.2408	
3	0.59	3	0.8420	1.1620	
3	0.59	4	0.8500	1.1731	
4	1.14		0.7500	1.0472	
4	1.14		1.0000	1.4588	
4	1.14	1 14	0.8950	1.2408	
4	1.14		0.9000	1.2490	*
5	2.18		0.8950	1.2408	
5 5	2.18		0.8950	1.2408	
5	2.18		0.7370	1.0323	
5	2.18		0.8950	1.2408	
6	4.32		0.6320	0.9190	
6	4.32		0.8000	1.1071	
6	4.32		0.7370	1.0323	
6	4.32		0.8000	1.1071	
7	8.81		0.2110	0.4773	
7	8.81		0.0500	0.2255	
7	8.81		0.0530	0.2323	
7	8.81		0.1000	0.3218	

t-test of Solvent and	Blank Controls	Ho:GRP1 MEAN = GRP2 MEAN
GRP1 (SOLVENT CRTL) MEAN = GRP2 (BLANK CRTL) MEAN = DIFFERENCE IN MEANS =	= - =	ALCULATED t VALUE = -0.1599 EGREES OF FREEDOM = 6

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05 TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

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.

413862-02, trifluralin, larval survival at termination
File: a:41386202.dt3 Transform: ARC SINE(SQUARE ROOT(Y))

## ANOVA TABLE

SOURCE	DF	SS	MS /	<b>F</b>
Between Within (Error)	6 21	2.599 0.294	0.433 0.014	30.948
Total	27	2.893		

Critical F value = 2.57 (0.05,6,21) Since F > Critical F REJECT Ho: All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	acetone control	1.154	0.822		
2	control	1.169	0.843	-0.179	
รั	0.59	1.206	0.872	-0.622	
A	1.14	1.249	0.886	-1.132	
5	2.18	1.189	0.856	-0.412	<u>.</u>
6	4.32	1.041	0.742	1.349	- <del>-</del>
7	8.81	0.314	0.103	10.042	*

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

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	acetone control	4			
2	control	4	0.176	21.4	-0.021
3	0.59	4	0.176	21.4	-0.050
	1.14	4	0.176	21.4	-0.064
5	2.18	4	0.176	21.4	-0.033
	4.32	4	0.176	21.4	0.080
6 7	8.81	4	0.176	21.4	0.719

Analysis of Variance		n weight	Date: 04-24-1992
N's, means and standard deviations	s based on	dependent varia	nbie: Weight
* Indicates statistics are colla	apsed over		nan /4 )
Factors: C R	N	Mean	S.D. RSD(%)
* *	396	0.4255	0.1368
1 *= actor south	, 6 <b>2</b>	0.4860	0.1333
2 *= leg den- etteren		0.4534	0.1565
3 ** ** ** ** ** ** ** ** ** ** ** ** **	68	0.4235	0.1253
4 *= 4	7.0	0.4365	0.1247
5 *= - >	65	0.4194	0.1140
6 *= 4.5+	58	0.3649	0.1029
7 *= 351	8	0.1393	0.0581
* 1	96	0.4110	0.1487
* 2	105	0.4327	0.1274
* 3	90	0.4220	0.1344
* 4	105	0.4346	0.1372
1 1	13	0.5874	0.1301 22.1
1 2	19	0.4768	0.0954 20.0
1 3	13	0.4774	0.1294 27.1
1 4	17	0.4252	0.1408 33.1
$\ddot{2}$ $\ddot{1}$	17	0.4042	0.1354
2 2	15	0.4380	0.1556
2 3	15	0.4292	0.1875
2 <b>4</b>	18	0.5328	0.1275
3 1	18	0.3822	0.1529
3 2	17	0.4462	0.1231
3 3	16	0.4121	0.1055
3 4	17	0.4554	0.1078
4 1	15	0.4354	0.0935
4 2	20	0.4374	0.1269
4 3	17	0.4263	0.1320
4 4	18	0.4462	0.1459
5 1	17	0.4132	0.1123
5 2	17	0.4358	0.1310
5 3	14	0.4013	0.1122
. 54	17	0.4240	0.1066
6 1	12	0.3315	0.0764
	16	0.3695	0.1093
6 2	14	0.4122	0.1092
6 3	16	0.3441	0.1012
6 4		0.1328	0.0339
7 1	4 1 1 2	0.1528	0.0000
7 2	1	0.1516	0.0000
7 3	, T		0.1317
7 4	2	0.1638	0.131/

Fmax for testing homogeneity of between subjects variances: Not defined						
Analysis of Va	riance	Depende	nt variable	: WEIGHT	1	
Source	df	SS (H)	MSS	F	<b>P</b>	•
Between Subject	ts 395	7.3915	•			
Ç (ÇONC)	· 6	1.1568	0.1928	12.358	0.0000	
R (REP)	3	0.0183	0.0061	0.390	0.7621	<u>s</u>
CR	18	0.4750	0.0264	1.691	0.0383	
Subi w Grour	<del></del> -	5.7414	0.0156			

Analysis of Variance Post-hoc tests for factor C (CONC) fish weight

Date: 04-24-1992

Level	Mean	Level	Mean
1	0.486	6	0.365
2	0.453	7	0.139
3	0.424	•	
4	0.437		
5	0.419	•	
		•	
		Bon-	

en e	Bon-	
Comparison 1 > 2	ferroni	Dunnett
1 > 3 1 > 4	0.0979	
1 > 5	0.0600	
1 > 6	0.0000 *	0.0100
1 > 7	0.0000*	0.0100
2 > 3		N.A.
2 > 4		N.A.
2 > 5		N.A.
2 > 6	0.0024 * *	
2 > 7	0.0000 * *	N.A.
3 < 4		N.A.
3 > 5		N.A.
3 > 6		N.A.
3 > 7	0.0000	N.A.
4 > 5	0.0000	N.A.
4 > 6	0.0288	N.A.
4 > 7	0.0000	N.A.
	0.0000	N.A.
5 > 6	0.0000	
5 > 7	0.0000	N.A.
6 > 7	0.0000	N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Va	riance	fish	length	Date: 04-24-19	9.2
N's, means and	standard deviation	s based on	dependent varia	DIE: LENGIH	,
* Indicates	statistics are coll	apsed over	this factor		
Factors:	C R	N	Mean	S.D.	
	* *	396	35.1591	4.1384	
	1 *= autor voitia	62	36.9516	3.7260	
T.	2 *= (1) tonetu.	65	36.0462	3.8707	
	3 *= こうきゃ/し **	68	35.6912	3.5334	
	4 *= / /	70	35.9286	3.3979	
\$	5 *= /	65	35.1231	3.1943	
	6 *= 4 *	58	32.4655	3.3204	
The second secon	7 *= 55	8	22.6250	2.8253	
	* 1	96	34.9167	4.6739	
	* 2	105	35.2762	4.0036	
	* 3	90	35.1222	3.7532	- *. 
	* 4	105	35.2952	4.1136	
	1 1	13	39.0000	3.1885	
	1 2	19	37.0000	2.4721	
•	1 3	13	36.5385	3.6427	
	1 4	17	35.6471	4.8081	
	2 1	17	35.2353	3.3640	
	2 2	15	35.6000	3.9424	
	2 3	15	34.8667	4.4056	
* * * * * * * * * * * * * * * * * * * *	2 4	18	38.1667	3.1854	
	3 1	18	35.6111	4.4740	
e de la companya del companya de la companya del companya de la co	3 2	17	35.8235	3.8768	
	3 3	16	35.0625	2.8395	
·	3 4	17	36.2353	2.7733	
	4 1	15	36.0000	2.7255	
N. C.	4 2	20	36.1500	4.3319	
	4 3	17	36.0000	3.2210	
	4 4	18	35.5556	3.1290	
	5 1	17	35.1765	2.4299	
	5 2	17	34.5882	4.0320	
	5 3	14	35.6429	3.0283	
	5 4	17	35.1765	3.2641	
	6 1	12	31.7500	3.5452	
		16	32.6250	3.4424	
	6 2 6 3	14	33.6429	2.9511	
		16	31.8125	3.3310	
		4	21.5000	1.7321	
e e	7 1	i	25.0000	0.0000	
•	7 2	1	20.0000	0.0000	
	7 3	2	25.0000	4.2426	
	7 4	.2	25.000	7.2720	

Fmax for testing homogeneity of between subjects variances: Not defined							
Analysis of Variance	Depende	ent variable	: LENGTH				
Source df	SS (H)	MSS	F	P			
Between Subjects 395	6764.9766	•				,	
C (CONC) 6	1988.7784	331.4631	27.175	0.0000			
R (REP) 3	3.1928	1.0643	0.087	0.9673		<b>.</b> .	
CR 18	284.3340	15.7963	1.295	0.1858	*		

4488.6709

368

Subj w Groups

12.1975

Date: 04-24-1992

```
Post-hoc tests for factor C (CONC)
                  Level
 Level
          Mean
                            Mean
          36.952
                           32.466
   1
                     6
                           22.625
   2
          36.046
                     7
   3
          35.691
          35.929
          35.123
   5
                     Bon-
                   ferroni
     Comparison
                            Dunnett
       1 > 2
       1 > 3
       1 > 4
                                          Linete & will
       1 > 5
                   0.0713 *
                   0.0000 ** 0.0100
       1 > 6
       1 > 7
                   0.0000 ** 0.0100
       2 > 3
                               N.A.
       2 > 4
                               N.A.
       2 > 5
                               N.A.
       2 > 6
                   0.0000 **
                               N.A.
       2 > 7
                   0.0000 **
                               N.A.
       3 < 4
                               N.A.
                               N.A.
        3 > 5
        3 > 6
                   0.0000
                               N.A.
                   0.0000
       3 > 7
                              N.A.
        4 > 5
                               N.A.
                   0.0000
                               N.A.
        4 > 6
        4 > 7
                   0.0000
                               N.A.
                               N.A.
        5 > 6
                   0.0008
        5 > 7
                   0.0000
                               N.A.
        6 > 7
                   0.0000
                               N.A.
```

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

fish length

Analysis of Variance

Shaughnessey # 036/0/	Chemical Name Trifluth Line Chemical Class Chemical Class	Page	/ of /
Study/Species/Lab/ Chemical X a.1.  Chronic Fish  \$\text{99.56}\$	Results Concentrations Tested (pp $b$ ) - $0.57$ , $1.14$ , $2.15$ , $4.57$ , $5.5$ ,	Reviewer/ V Date	Validation Status
Species: Salme gundari MATC = > 114 < x  Lab: Lilly Research Lubraturia Effected Parameters	MATC = > 1.14 < 2.15 pp $\frac{1}{4}$ .  Effected Parameters = $\frac{4^{3}k_{11}q_{11}l_{11}}{5l_{11}l_{11}l_{11}}$ $\frac{1}{4^{2}l_{11}l_{11}l_{11}}$ $\frac{1}{4^{2}l_{11}l_{11}l_{11}}$	2,141h	lire
MRID # 413863-63	Control Mortality (%) - Solvent Control Mortality (%) - Comments: ** Much much link link hins  (A) Most Sens, hoe purameter		
Chronic Invertebrate	Concentrations Tested (pp) =		
Species: Lab:	MATC = > < Pp  Effected Parameters =		
MRID #	<pre>Control Mortality (%) - Solvent Control Mortality (%) - Comments:</pre>		

Date: 04-24-1992

Raw Data

Data listing Obs. CONC 1 1 2 1 3 1 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	g REP WEIGHT 1 0.3200 1 0.5810 1 0.5810 1 0.5557 1 0.55595 1 0.55712 1 0.6541 1 0.6352 1 0.6541 1 0.6357 1 0.6547 2 0.4768 2 0.4776 2 0.4780 2 0.4989 2 0.4165 2 0.4989 2 0.4165 2 0.5711 2 0.5884 2 0.6101 2 0.4021 2 0.5884 2 0.6735 2 0.5314 2 0.5314 2 0.5884 2 0.6735 3 0.6231 3 0.6231 3 0.5828 3 0.4380 3 0.3979 3 0.4615 4 0.4615 4 0.4615 4 0.4615 4 0.4835	

Oha	CÓNG	משמ	METCHE	TENCHU
Obs.	CONC	REP	WEIGHT	LENGTH
56	1	4	0.6617 0.3288	38
57	1	4		24
58	1	4	0.6019	34
59	1	4	0.4127	38
60	1	4	0.1245	40
61	1	4	0.4036	35
62	1	4	0.6188	32
63	2	1	0.5828	37
64	. 2	1	0.1695	33
65	2	1	0.4811	38
66	2	1	0.4854	34
67	2	1	0.4994	39
68	2	1	0.3335	37
69	2	1	0.2831	38
70	2	1	0.4662	32
71	2	.1	0.3382	30
72	2	1	0.6097	39 34
7.3	2	1	0.6027	34
74	2	1	0.4826	28 32
75	2	1	0.2686	32
76	. 2	1	0.3287	39
77	2	1	0.2877	36
78	2	1	0.2275	35
79	2	1	0.4249	38
8.0	2	2	0.5348	36
81	2	2	0.3229	31
82	2	2	0.6492	33
83	2	2	0.4800	34
84	<sup>1</sup> /- 2	2	0.4860	31
85	2	2	0.6186	41 37 40
86	2	2	0.2768	37
87	. 2	2	0.2293	40
88	2	2	0.4131	31
89	2 2 2 2 2 2	2 2 2	0.3322	34
90	2	2	0.3635	38
91	2	2	0.2916	42
92		2	0.7836	37
93	. 2	2	0.4338	30
94	2	2 2 2	0.3539	39
95	2	3	0.8085	43
96	2	3 3	0.6003	36
97	2	,3	0.4138	34
98	2	3	0.3112	35
99	2	3	0.2918	31
100	2	3	0.4348	33
101	2	3	0.4547	33 29 32
102	2	3	0.3388	32
103	2	3	0.2357	31
104	2	3	0.1884	38
105	2	3	0.4312	41
106	2	3		41
107	2	3	0.5946	29
108	2	3	0.7557	33
109		3	0.2234	31 38 41 41 29 33 37 41
110	) 2	4	0.4301	41
1,10		7	U. 1001	• •

Obs.	CONC	REP	WE	ΪG	HT	LEN	GTH
111		4			39		42
	2 2						41
112	2	4			64		
113	2	4			32		42
114	2	4			27		39
115	2	4	0.	48	69		38
116	2 2 2 2	4	0.	60	60		41
117	2	4			20		3.2
118	2	4			33	•	32
119	2	4			42		36
112	2						35
120		4			92		35
121	2	4			556		37
122	2	4			10		39
123	2	4	0.	71	42		34
124	2	4	0.	32	20		40
125	2 2 2	4			46		39
126	2	4	0.	68	374		40
127	2	4	0.	48	377		39
128	3	1			708		41
	3	ī			528		42
129	3		Ο.		700		36
130	3	1	.0 ,	. D /	708		39
131	3	1			316		39
132	3 3	1			342		32 29
133	- 3	1			149		29
134	3	1	0.	. 2:	L40		38
135	3	1	0.	. 22	277		34
136	3	1	0.	. 18	366		42
137	. 3	1			101		38
138	3 3 3 3 3 3 3 3 3 3 3	1			75		33
120		ī			543		32
139	3				439		20
140	3	1					28 37 38 37 37 28
141	3	1			273		3/
142	3	1	0		392		38
143	3	1	0		911		37
144	3	. 1	0		973		37
145	3	ī	0	.39	961		28
146	3	2	0	. 4	092		33
147	3				724		36
148	3	2	ñ		042		41
149		2	Õ		240		36
	3 3	2 2 2 2	0		016		29
150	3	. 2	0				25
151	3	2	U		238		35
152	3	2	O		107		36
153	3	2	0		473		39
154	3 3 3 3	2	. 0	. 4	104	:	34
155	. 3	2	0	. 4	291		26
156	3	2	0		000		36
157		2	0		753		39
157		. 2			, 53 219		38
158		2	. •				3 5
159	3	2	Ü		231		33
160	3	3 2	. 0		368		38 35 37 38 41
161	. 3	,2	0		233		38
162		2	: 0	. 3	719	•	41
163				. 3	860	)	34
164		3		. 3	609	•	34
					284		38
165	. 3	, , .	, 0		204	=	20

Obs.	CONC	REP	WEIGHT	LENGTH
166	3	3	0.3062	43
167	3	3	0.2936	36
168 169	3	3	0.5042 0.3304	38 36
170	3	3	0.5364	35
171	3	.3	0.3472	34
172	-3	3	0.4991	34
173	3 3	3	0.3032 0.4324	34 33
174 175	3	· 3	0.4324	32
176	3	. 3	0.3662	32
177	, 3	3	0.4562	32
178	3	3	0.4824	36
179 180	3	4	0.1818 0.5208	38 37
181	3	4	0.5233	36
182	3	4	0.3924	36
183	3	4	0.3928	37
184	. 3	4	0.3992	36
185 186	3 3	4	0.6094 0.4578	36 27
187	3	4	0.4852	37
188	3	4	0.5718	37
189	3	4	0.6113	34
190 191	3	4	0.4553 0.4939	35 39
192	3	4	0.5129	37
193	3	4	0.4252	38
194	3	4	0.2992	40
195 196	3	4 1	0.4283 0.5136	36 40
197	4	ī	0.5024	34
198	4	1	0.4598	32
199	4	1	0.5026	35
200 201	4	1 1	0.4733 0.5604	37 37
202	4	1	0.2781	35
203	4	1	0.5774	31
204	4	,1	0.4635	39
205	4	1	0.4112	34 34
206 207	4 4	1	0.3394	39
208	4	ī	0.3078	38
209	4	1	0.3045	39
210	4	1	0.4225	36
211 212	4 4	2 2	0.0746	37 28
213	4	2	0.4577	38
214	4	2	0.4422	40
215	4	.2	0.4831	33
216	4	2	0.4313	37 36
217 218	4	2 2	0.1773	37
219	4	2	0.4570	
220	4	2	0.5843	35

)ha	CONC	משם	we	IGI	TT.	LENC	יחינו
obs. 221	4	REP 2		47		DLING	38
222	4	2		38			40
223	4	2		51			37
224	4	2		55			22
225	4	2		39			37
226	4	2		57			37
227	4	2		53			40
228	4	2		42			38
229	4	2	0.	49	80		35
230	4	2	0.	32	25		40
231	4	3	0.	44	38		34
232	4	3		44			35
233	4	3		51			38
234	4	3		55			31
235	4	3		62		·r	35
236	4	3		56			38
237	4	3		47			38 34
238 239	4	3		17 35			37
239	4	3		58			40
241	4	3		28			40
242	4	3		26			32
243	4	3		57			40
244	4	3		35			41
245	4	3		32			34
246	4	3		30			3.2
247	4	3		38			33
248	4	4		41			28
249	4	4		54			35
250	4	4		61			33
251	4	4		45			35
252	4	4		. 39 . 32			40 38
253 254	4	4 4		. 32 . 34			35
255	4	4		. 85			34
256	4	4		. 57			31
257	4	4		. 27			35
258	4	4		. 52			37
259	4	4		. 48			39
260	4	4		. 36			38
261	4	4	0	. 20	79		33
262	4			. 37			34
263	4			. 46			39
264	4			. 36			38
265	4	4		. 45			38
266	5			.37			38
267	5			. 39			34
268	5			.35 .51			34 29
269	5 5			. 65			38
270 271				. 54			34
271	5 5			. 22			37
273				.33			39
274				.34			34
275				. 48			33

Obs.	CONC	REP	WEIGHT	LENGTH
276	5	1	0.3302	36
277 278	5 5	1	0.3410	35 38
279	5	1	0.4366	35
280	5	ī	0.4036	36
281	5	1	0.5985	34
282	5.	1	0.3511	34
283	5	2	0.6452	32
284 285	5 5	2	0.1784 0.4350	36 35
286	5	2	0.3399	25
287	5	2	0.4202	36
288	5	2	0.5895	38
289	5	2	0.4100	26 35
290 291	5 5	2	0.5626	38
292	5	2	0.4065	32
293	. 5	2	0.5261	3,5
294	5	2	0.4201	38
295	5	2	0.3822	35
296 297	5 5	2	0.6340 0.1940	34 40
298	5	2	0.4712	38
299	5	2	0.3832	35
300	5	3	0.3018	40
301	5	3	0.4507	34
302 303	5 5	3 3	0.4940 0.3694	38 33
304	5	3	0.3831	30
305	5	3	0.3492	37
306	5	3	0.4435	35
307	5 5	3	0.1982 0.3792	42 36
308 309	5 5	3	0.3792	36
310	. 5	3	0.3293	36
311	5	3	0.4946	34
312	5	3	0.6790	33
313	5	3	0.4080	35
314 315	5 5	4	0.4213 0.3665	38 33
316	5	4	0.4254	37
317	5	4	0.4962	36
318		4	0.5218	38
319	5	4	0.5505	38
320 321	5 5	4 4	0.3384	32 33
322	5 5	4	0.4990	36
323	5	-4	0.2858	36
324	5	4	0.5730	
325	5	4		
326	. 5 5	. 4 . 4		
327 328	. 5			
329	5			
330	5			

Obs.	CONC	REP	WEIGHT	LENGTH
331	6	1	0.3493	34
332	6	1	0.1330	3.6
333	6	1	0.2972	22
334	6	1	0.3376	32
335	6	1	0.3452	31
336 337	6 6	1	0.3508 0.3558	32 34
338	6	1	0.3338	33
339	6	ī	0.3124	33
340	6	1	0.3938	30
341	6	1	0.4028	30
342	6	1	0.4276	34
343 344	6	2	0.4092 0.2556	37 36
345	6	2	0.6113	40
346	6	2	0.3652	33
347	6	2	0.5097	34
348	. 6	2	0.2858	34
349	6	2	0.2748	30
350	6	2	0.4525 0.3646	29 32
351 352	6	2 2	0.3646	34
353	6	2	0.3692	31
354	6	2	0.2934	31
355	6	2	0.3868	31
356	6	2	0.3067	25 23
357 358	6 6	2 2	0.1748 0.3572	33 32
359	6	3	0.5460	34
360	6	3	0.6156	28
361	6	3	0.4027	30
362	6	3	0.2757	33
363	6	3	0.5971	31
364 365	6 6	3	0.4757	38 32
366	6	3	0.3213	36
367	6	3	0.3762	39
368	6	3	0.3888	35
369	6	3	0.2996	
370		3	0.3308	
371	6	3	0.3680	
372 373	6		0.3311	
374			0.2300	
375			0.3798	
376				
377			0.4166	
378				
379				
380 381				
382				
383				28
384	. 6			
385	6	4	0.2021	. 28

Obs.	CONC	REP	WEIGHT	LENGTH
386	6	4	0.4183	35
387	6	4	0.4294	29
388	6	4	0.2949	
389	7	i	0.1012	32
390	7	1	0.1012	20
391	7	1		20
392	7		0.1790	23
393	•	1	0.1358	23
	7	2	0.1518	25
394	7	3	0.1034	20
395	7.	4	0.2570	22
396	7	4	0.0707	28