

US EPA ARCHIVE DOCUMENT

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 SUBMISSION \_\_\_\_\_ 02-12-90

DATE RECEIVED BY EFED \_\_\_\_\_ 02-25-92

SRRD/RD REQUESTED COMPLETION DATE \_\_\_\_\_ 05-24-92

EEB ESTIMATED COMPLETION DATE \_\_\_\_\_ 05-24-92

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 F R I N H D \_\_\_\_\_ Herbicide

COMPANY NAME \_\_\_\_\_ DowElanco

SUBMISSION PURPOSE \_\_\_\_\_ Review fish early life stage and

INCLUDE USE(S) \_\_\_\_\_ aquatic invertebrate life cycle  
\_\_\_\_\_ studies

COMMON CHEMICAL NAME \_\_\_\_\_ Trifluralin

DP BARCODE: D174857

REREG CASE #

CASE: 818802  
SUBMISSION: S412176

DATA PACKAGE RECORD  
BEAM 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 703-308-8062 ROOM: CS1 383  
PM TEAM REVIEWER: TERRI STOWE 703-308-8043 ROOM: CS1 3D5  
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 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	02 125 92	1 1
BRAN: EEB	02 126 92	1 1 - 7/16/92
SECT:	1 1	1 1
REVR :	1 1	1 1
CONTR:	1 1	1 1

\*\*\* 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 adequate data have not been submitted to the Agency.

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

DP BC BRANCH/SECTION DATE OUT DUE BACK INS CSF LABEL

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. Daphnia magna:

This study is scientifically sound and meets 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 ppb based on mean measured concentration, the highest concentration tested.

*Curtis E. Laird 6-30-92*

Curtis E. Laird, Fishery Biologist  
Ecological Effects Branch  
Environmental Fate and Effects Division (H7507C)

*Allen W. Vaughan 7-14-92*

Allen Vaughan, Acting Head-Section 2  
Ecological Effects Branch  
Environmental Fate and Effects Division (H7507C)

*Douglas J. Urban 7/10/92*

Douglas J. Urban, Acting Chief  
Ecological Effects Branch  
Environmental Fate and Effects Division (H7507C)

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Trifluralin.  
Shaughnessey No. 036101.
2. **TEST MATERIAL:** 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. **STUDY TYPE:** *Daphnia magna* Life-Cycle (21-Day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.
4. **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. 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:
Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA	Signature:  Date:

C.E.L. 6-29-92  
AWV 7.14.92
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  $\mu\text{g/l}$  based on mean measured concentration, the highest concentration tested.

4

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5. **REVIEWED BY:**

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

Signature: *Louis M. Rifici*

*5/22/92*

Date:

6. **APPROVED BY:**

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

Signature: *P. Kosalwat*

*5/22/92*

Date:

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

Signature: *H.T. Craven*

*CEL 6-29-92*

*7/15/92*

Date:

7. **CONCLUSIONS:** 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*. 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  $\mu\text{g/l}$  mean measured concentration, the highest concentration tested.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

*12.25*

*5*

8. **RECOMMENDATIONS:** 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* ( $\leq 24$  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  $20 \pm 2^\circ\text{C}$  in conditioned well water having a total hardness of 103-120 mg/l as  $\text{CaCO}_3$ , a total alkalinity of 120-140 mg/l as  $\text{CaCO}_3$ , and a conductivity of  $230 \mu\text{S/cm}$ . 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  $20^\circ\text{C}$  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  $300 \mu\text{l}$  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.

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C. **Dosage:** 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  $\mu\text{g}/\text{l}$ ), an acetone control (0.1 ml/l), and a dilution water control were selected for the test.

D. **Design:** 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. Statistics:** 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

$\mu\text{g}/\text{l}$ . 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  $\mu\text{g}/\text{l}$  exposure on day 12. Temporary hypoactivity was observed in at least one daphnid from the 6.53, 13.7, and 50.7  $\mu\text{g}/\text{l}$  exposures. Ten percent immobilization was observed at 3.19, 13.7, and 26.2  $\mu\text{g}/\text{l}$  and 20% immobilization occurred at 50.7  $\mu\text{g}/\text{l}$ . "This immobilization does not appear to be related to increasing concentrations of trifluralin."

Reproduction was significantly decreased at 13.7  $\mu\text{g}/\text{l}$  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  $\text{CaCO}_3$ , 145-155 mg/l as  $\text{CaCO}_3$ , and 232-291  $\mu\text{S}/\text{cm}$ , respectively. The concentration of un-ionized ammonia was  $<0.001$  mg/l.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The chronic no-observed-effect concentration of trifluralin for *Daphnia magna* was 50.7  $\mu\text{g}/\text{l}$ .

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. **Test Procedure:** 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  $\mu\text{g}/\text{l}$  was significantly affected. The reviewer's comparison of reproduction at 13.7  $\mu\text{g}/\text{l}$  to that of the solvent control indicated no significant difference (see attached printout 3).
- 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  $\mu\text{g}/\text{l}$  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  $\mu\text{g/l}$  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 tested (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.

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Page \_\_\_\_\_ is not included in this copy.

Pages 12 through 15 are not included.

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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - 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.
  - FIFRA registration data.
  - The document is a duplicate of page(s) \_\_\_\_\_.
  - The document is not responsive to the request.
- 

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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TITLE: 413862-01, Trifluralin, total young per female  
 FILE: a:41386201.dtl  
 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
1	acetone control	6	97.0000	97.0000
1	acetone control	7	91.0000	91.0000
1	acetone control	8	141.0000	141.0000
1	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
3	1.57	1	94.0000	94.0000
3	1.57	2	119.0000	119.0000
3	1.57	3	61.0000	61.0000
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
4	3.19	4	78.0000	78.0000
4	3.19	5	96.0000	96.0000
4	3.19	6	138.0000	138.0000
4	3.19	7	64.0000	64.0000
4	3.19	8	82.0000	82.0000
4	3.19	9	150.0000	150.0000

TITLE: 413862-01, Trifluralin, total young per female  
 FILE: a:41386201.dtl  
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

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

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

Bartlett's test for homogeneity of variance  
 Data PASS homogeneity test at 0.01 level. Continue analysis.

413862-01, Trifluralin, total young per female

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN = 104.5000      CALCULATED t VALUE = -1.1192  
 GRP2 (BLANK CRTL) MEAN = 120.4444      DEGREES OF FREEDOM = 17  
 DIFFERENCE IN MEANS = -15.9444

TABLE t VALUE (0.05 (2),17) = 2.110      NO significant difference at alpha=0.05  
 TABLE t VALUE (0.01 (2),17) = 2.898      NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	24176.683	3453.812	3.927
Within (Error)	65	57161.344	879.405	
Total	72	81338.027		

Critical F value = 2.17 (0.05,7,60)  
 Since F > Critical F REJECT Ho:All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	acetone control	104.500	104.500		
2	dilut. control	120.444	120.444	-1.170	
3	1.57	97.400	97.400	0.535	
4	3.19	102.111	102.111	0.175	
5	6.53	85.111	85.111	1.423	
6	13.7	70.889	70.889	2.467	
7	26.2	114.778	114.778	-0.754	
8	50.7	134.000	134.000	-2.097	

Bonferroni T table value = 2.52 (1 Tailed Value, P=0.05, df=60,7)

BONFERRONI T-TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	acetone control	10			
2	dilut. control	9	34.391	32.9	-15.944
3	1.57	10	33.473	32.0	7.100
4	3.19	9	34.391	32.9	2.389
5	6.53	9	34.391	32.9	19.389
6	13.7	9	34.391	32.9	33.611
7	26.2	9	34.391	32.9	-10.278
8	50.7	8	35.504	34.0	-29.500

TITLE: 413862-01, Trifluralin, daphnid length at termination  
 FILE: a:41386201.dt2  
 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	acetone control	1	4.5400	4.5400
1	acetone control	2	4.6600	4.6600
1	acetone control	3	4.1400	4.1400
1	acetone control	4	4.5800	4.5800
1	acetone control	5	4.3600	4.3600
1	acetone control	6	4.3000	4.3000
1	acetone control	7	4.3000	4.3000
1	acetone control	8	4.3200	4.3200
1	acetone control	9	4.1400	4.1400
1	acetone control	10	4.2600	4.2600
2	dilut. control	1	4.5000	4.5000
2	dilut. control	2	4.6600	4.6600
2	dilut. control	3	4.6600	4.6600
2	dilut. control	4	4.5800	4.5800
2	dilut. control	5	4.5000	4.5000
2	dilut. control	6	4.5800	4.5800
2	dilut. control	7	4.3000	4.3000
2	dilut. control	8	4.3200	4.3200
2	dilut. control	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
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	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

5	6.53	1	4.4400	4.4400
5	6.53	2	4.4400	4.4400
5	6.53	3	4.5000	4.5000
5	6.53	4	4.3600	4.3600
5	6.53	5	4.5800	4.5800
5	6.53	6	4.5000	4.5000
5	6.53	7	4.4800	4.4800
5	6.53	8	4.6200	4.6200
5	6.53	9	4.5400	4.5400
6	13.7	1	4.5400	4.5400
6	13.7	2	4.5400	4.5400
6	13.7	3	4.6200	4.6200
6	13.7	4	4.4400	4.4400
6	13.7	5	4.4800	4.4800
6	13.7	6	4.6200	4.6200
6	13.7	7	4.5800	4.5800
6	13.7	8	4.3600	4.3600
6	13.7	9	4.3200	4.3200
7	26.2	1	4.4000	4.4000
7	26.2	2	4.4800	4.4800
7	26.2	3	4.4800	4.4800
7	26.2	4	4.6200	4.6200
7	26.2	5	4.5000	4.5000
7	26.2	6	4.5000	4.5000
7	26.2	7	4.5400	4.5400
7	26.2	8	4.5000	4.5000
7	26.2	9	4.2200	4.2200
8	50.7	1	4.7200	4.7200
8	50.7	2	4.5000	4.5000
8	50.7	3	4.5800	4.5800
8	50.7	4	4.3600	4.3600
8	50.7	5	4.6200	4.6200
8	50.7	6	3.7200	3.7200
8	50.7	7	4.6600	4.6600
8	50.7	8	4.6600	4.6600

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

Bartlett's test for homogeneity of variance  
 Data FAIL homogeneity test at 0.01 level. Try another transformation.

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

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

-----  
 GRP1 (SOLVENT CRTL) MEAN = 4.3600 CALCULATED t VALUE = -1.3507  
 GRP2 (BLANK CRTL) MEAN = 4.4711 DEGREES OF FREEDOM = 17  
 DIFFERENCE IN MEANS = -0.1111  
 -----

TABLE t VALUE (0.05 (2),17) = 2.110 NO significant difference at alpha=0.05  
 TABLE t VALUE (0.01 (2),17) = 2.898 NO significant difference at alpha=0.01

-----  
 WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	acetone control	4.360				
2	dilut. control	4.471	105.50	60.00	9	
3	1.57	4.358	103.50	72.00	10	
4	3.19	4.496	110.50	60.00	9	
5	6.53	4.496	111.50	60.00	9	
6	13.7	4.500	112.50	60.00	9	
7	26.2	4.471	105.50	60.00	9	
8	50.7	4.478	96.00	48.00	8	

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

## SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	control	10	0	
2	1.57	10	0	
3	3.19	10	1	
4	6.53	10	0	
5	13.7	10	1	
6	26.2	10	1	
7	50.7	10	2	

Study/Species/Lab/ Chronic Fish Chemical MRID # \_\_\_\_\_ % a.i. \_\_\_\_\_ Results \_\_\_\_\_ Reviewer/ \_\_\_\_\_ Validation \_\_\_\_\_ Date \_\_\_\_\_ Status \_\_\_\_\_

Concentrations Tested (ppm) - \_\_\_\_\_  
\_\_\_\_\_

Species: MATC - > \_\_\_\_\_ < \_\_\_\_\_ ppb \_\_\_\_\_

Lab: Effected Parameters - \_\_\_\_\_

Control Mortality (%) - \_\_\_\_\_ Solvent Control Mortality (%) - \_\_\_\_\_

MRID # \_\_\_\_\_ Comments: \_\_\_\_\_

Chronic Invertebrate 99.86 Concentrations Tested (ppb) - 1.57, 2.19, 6.53, 13.1, 20.2, 50.7

Species: Daphnia magna MATC - > 50.7 < \_\_\_\_\_ ppb \_\_\_\_\_

Lab: Lilly Research Laboratories Effected Parameters - None

Control Mortality (%) - 0 Solvent Control Mortality (%) - 0

MRID # 413562-01 Comments: \* Mean measured concentration

LALC Supplemental  
7/25/77

pw

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Trifluralin.  
Shaughnessey No. 036101.
- 2. **TEST MATERIAL:** Trifluralin (compound 036352);  $\alpha, \alpha, \alpha$ -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; Lot No. 326EF8; 99.86% active ingredient.
- 3. **STUDY TYPE:** Freshwater Fish, Early Life-Stage Flow-Through Toxicity Test. Species Tested: Rainbow Trout (*Salmo gairdneri*).
- 4. **CITATION:** Adams, E.R., P.J. Cocke, and M.D. Gunnoe. 1990. 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. EPA MRID No. 413862-02.

5. **REVIEWED BY:**

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

Signature: *Louis M. Rifici*  
Date: *5/22/92*

6. **APPROVED BY:**

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

Signature: *P. Kosalwat*  
Date: *5/22/92*

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

Signature: *Henry T. Craven*  
Date:

- 7. **CONCLUSIONS:** 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 MATC was  $>1.14 \mu\text{g/l}$  and  $<2.18 \mu\text{g/l}$  mean measured concentrations (geometric mean MATC =  $1.58 \mu\text{g/l}$ ).
- 8. **RECOMMENDATIONS:** N/A.

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  $\text{CaCO}_3$ , an alkalinity of 125 mg/l as  $\text{CaCO}_3$ , a conductivity of 220  $\mu\text{S}/\text{cm}$ , 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  $\mu\text{g}/\text{l}$ , 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  $\text{CO}_2$  (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  $\mu\text{E}/\text{m}^2/\text{sec}$ .

C. **Dosage:** 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  $\mu\text{g/l}$ ), a dilution water control, and a solvent control (0.02-0.1 ml acetone/l) were used.

D. **Design:** 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. **Statistics:** 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  $\mu\text{g}/\text{l}$ . 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  $\mu\text{g}/\text{l}$  was significantly reduced compared to the pooled controls (Table 4, attached).

No treatment-related behavioral effects were observed in larvae exposed to test concentrations  $\leq 4.32 \mu\text{g}/\text{l}$  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  $\geq 2.18 \mu\text{g}/\text{l}$  was significantly lower than that of the pooled control (Table 4, attached). Body weight was significantly reduced at trifluralin concentrations  $\geq 4.32 \mu\text{g}/\text{l}$  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  $\mu\text{g}/\text{l}$  test solutions averaged 196  $\mu\text{S}/\text{cm}$ , 128 mg/l as  $\text{CaCO}_3$ , and 153 mg/l as  $\text{CaCO}_3$ , respectively.

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

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. Test Procedure: 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  $\geq 4.32 \mu\text{g/l}$  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 ( $1.14 \mu\text{g/l}$  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.

- C. 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  $>1.14 \mu\text{g/l}$  and  $<2.18 \mu\text{g/l}$  mean measured concentrations (geometric mean MATC =  $1.58 \mu\text{g/l}$ ).

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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Page \_\_\_\_\_ is not included in this copy.

Pages 30 through 35 are not included.

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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - 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.
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TITLE: 413862-02, trifluralin, days to hatch  
 FILE: a:41386202.dtl  
 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
1	acetone control	3	8.0000	8.0000
1	acetone control	4	8.0000	8.0000
2	control	1	8.0000	8.0000
2	control	2	11.0000	11.0000
2	control	3	8.0000	8.0000
2	control	4	10.0000	10.0000
3	0.59	1	9.0000	9.0000
3	0.59	2	11.0000	11.0000
3	0.59	3	10.0000	10.0000
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
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	1	8.0000	8.0000
7	8.81	2	8.0000	8.0000
7	8.81	3	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.

Bartlett's test for homogeneity of variance  
 Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	11.357	1.893	1.500
Within (Error)	21	26.500	1.262	
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

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	acetone control	9.000	9.000		
2	control	9.250	9.250	-0.315	
3	0.59	10.000	10.000	-1.259	
4	1.14	8.500	8.500	0.629	
5	2.18	9.000	9.000	0.000	
6	4.32	9.000	9.000	0.000	
7	8.81	7.750	7.750	1.574	

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	1.954	21.7	-0.250
3	0.59	4	1.954	21.7	-1.000
4	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	1	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
5	2.18	1	1.0000	1.4588
5	2.18	2	0.9500	1.3453
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
6	4.32	3	0.9500	1.3453
6	4.32	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.

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

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	

Critical values use k = 6, are 1 tailed, and alpha = 0.05

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	2	0.9500	1.3453
1	acetone control	3	0.7220	1.0154
1	acetone control	4	0.8950	1.2408
2	control	1	0.8950	1.2408
2	control	2	0.7890	1.0935
2	control	3	0.7890	1.0935
2	control	4	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	1	0.7500	1.0472
4	1.14	2	1.0000	1.4588
4	1.14	3	0.8950	1.2408
4	1.14	4	0.9000	1.2490
5	2.18	1	0.8950	1.2408
5	2.18	2	0.8950	1.2408
5	2.18	3	0.7370	1.0323
5	2.18	4	0.8950	1.2408
6	4.32	1	0.6320	0.9190
6	4.32	2	0.8000	1.1071
6	4.32	3	0.7370	1.0323
6	4.32	4	0.8000	1.1071
7	8.81	1	0.2110	0.4773
7	8.81	2	0.0500	0.2255
7	8.81	3	0.0530	0.2323
7	8.81	4	0.1000	0.3218

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	1.1542	CALCULATED t VALUE =	-0.1599
GRP2 (BLANK CTRL) MEAN =	1.1692	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-0.0150		

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.

Bartlett's 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	F
Between	6	2.599	0.433	30.948
Within (Error)	21	0.294	0.014	
Total	27	2.893		

Critical F value = 2.57 (0.05,6,21)  
 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	acetone control	1.154	0.822		
2	control	1.169	0.843	-0.179	
3	0.59	1.206	0.872	-0.622	
4	1.14	1.249	0.886	-1.132	
5	2.18	1.189	0.856	-0.412	
6	4.32	1.041	0.742	1.349	
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
4	1.14	4	0.176	21.4	-0.064
5	2.18	4	0.176	21.4	-0.033
6	4.32	4	0.176	21.4	0.080
7	8.81	4	0.176	21.4	0.719

Analysis of Variance fish weight Date: 04-24-1992

N's, means and standard deviations based on dependent variable: WEIGHT

\* Indicates statistics are collapsed over this factor

Factors: C R	N	Mean	S.D.	RSD (%)
* *	396	0.4255	0.1368	
1 * = acetone solution	62	0.4860	0.1333	
2 * = heptane solution	65	0.4534	0.1565	
3 * = ...	68	0.4235	0.1253	
4 * = ...	70	0.4365	0.1247	
5 * = ...	65	0.4194	0.1140	
6 * = 4.5%	58	0.3649	0.1029	
7 * = 5.5%	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
2 1	17	0.4042	0.1354	
2 2	15	0.4380	0.1556	
2 3	15	0.4292	0.1875	
2 4	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	
5 4	17	0.4240	0.1066	
6 1	12	0.3315	0.0764	
6 2	16	0.3695	0.1093	
6 3	14	0.4122	0.1092	
6 4	16	0.3441	0.1012	
7 1	4	0.1328	0.0339	
7 2	1	0.1518	0.0000	
7 3	1	0.1034	0.0000	
7 4	2	0.1638	0.1317	

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance Dependent variable: WEIGHT

Source	df	SS (H)	MSS	F	P
Between Subjects	395	7.3915			
C (CONC)	6	1.1568	0.1928	12.358	0.0000
R (REP)	3	0.0183	0.0061	0.390	0.7621
CR	18	0.4750	0.0264	1.691	0.0383
Subj w Groups	368	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		

Comparison	Bon- ferroni	Dunnett
1 > 2		
1 > 3	0.0979	
1 > 4		
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 * *	N.A.
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		N.A.
4 > 6	0.0288	N.A.
4 > 7	0.0000	N.A.
5 > 6		N.A.
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).

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Analysis of Variance

fish length

Date: 04-24-1992

N's, means and standard deviations based on dependent variable: LENGTH

\* Indicates statistics are collapsed over this factor

Factors: C R	N	Mean	S.D.
* *	396	35.1591	4.1384
1 * = <i>autumn</i>	62	36.9516	3.7260
2 * = <i>winter</i>	65	36.0462	3.8707
3 * = <i>spring</i>	68	35.6912	3.5334
4 * = <i>summer</i>	70	35.9286	3.3979
5 * = <i>...</i>	65	35.1231	3.1943
6 * = <i>...</i>	58	32.4655	3.3204
7 * = <i>...</i>	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
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
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
6 2	16	32.6250	3.4424
6 3	14	33.6429	2.9511
6 4	16	31.8125	3.3310
7 1	4	21.5000	1.7321
7 2	1	25.0000	0.0000
7 3	1	20.0000	0.0000
7 4	2	25.0000	4.2426

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance

Dependent 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
CR	18	284.3340	15.7963	1.295	0.1858
Subj w Groups	368	4488.6709	12.1975		

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

fish length

Date: 04-24-1992

Level	Mean	Level	Mean
1	36.952	6	32.466
2	36.046	7	22.625
3	35.691		
4	35.929		
5	35.123		

Comparison	Bon- ferroni	Dunnnett
1 > 2		
1 > 3		
1 > 4		
1 > 5	0.0713*	
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	0.0000**	N.A.
2 > 7	0.0000**	N.A.
3 < 4		N.A.
3 > 5		N.A.
3 > 6	0.0000	N.A.
3 > 7	0.0000	N.A.
4 > 5		N.A.
4 > 6	0.0000	N.A.
4 > 7	0.0000	N.A.
5 > 6	0.0008	N.A.
5 > 7	0.0000	N.A.
6 > 7	0.0000	N.A.

*← not p value*

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

Study/Species/Lab/ MRID # \_\_\_\_\_ Chemical % a.i. \_\_\_\_\_ Results \_\_\_\_\_ Reviewer/ Date \_\_\_\_\_ Validation Status \_\_\_\_\_

Chronic Fish \_\_\_\_\_ Concentrations Tested (pp**b**) - 0.59, 1.14, 2.15, 4.30, 8.51

Species: Salmo gairdneri MATC - > 1.14 < 2.15 pp**b**. 99.56

Effected Parameters - (A) length at termination, weight at term.

Lab: Lilly Research Laboratories Survival at termination

Control Mortality (%) - \_\_\_\_\_ Solvent Control Mortality (%) - \_\_\_\_\_

MRID # 413862-02 Comments: \* mean measured (see concentrations)

(A) most sensitive parameter

Chronic Invertebrate \_\_\_\_\_ Concentrations Tested (pp\_\_\_\_) - \_\_\_\_\_

Species: \_\_\_\_\_ MATC - > \_\_\_\_\_ < \_\_\_\_\_ pp\_\_\_\_.

Lab: \_\_\_\_\_ Effected Parameters - \_\_\_\_\_

Control Mortality (%) - \_\_\_\_\_ Solvent Control Mortality (%) - \_\_\_\_\_

MRID # \_\_\_\_\_ Comments: \_\_\_\_\_

## Data listing

## Raw Data

Date: 04-24-1992

Obs.	CONC	REP	WEIGHT	LENGTH
1	1	1	0.3200	41
2	1	1	0.5810	40
3	1	1	0.7357	43
4	1	1	0.5221	40
5	1	1	0.5595	39
6	1	1	0.5712	38
7	1	1	0.3682	40
8	1	1	0.7539	31
9	1	1	0.6541	35
10	1	1	0.7147	38
11	1	1	0.5638	43
12	1	1	0.6352	40
13	1	1	0.6574	39
14	1	2	0.3768	40
15	1	2	0.4975	37
16	1	2	0.4776	35
17	1	2	0.3457	35
18	1	2	0.4180	37
19	1	2	0.4989	32
20	1	2	0.4165	38
21	1	2	0.5711	36
22	1	2	0.5419	38
23	1	2	0.3244	37
24	1	2	0.4827	39
25	1	2	0.4251	34
26	1	2	0.6101	33
27	1	2	0.4021	38
28	1	2	0.5884	37
29	1	2	0.6735	42
30	1	2	0.5314	38
31	1	2	0.3711	40
32	1	2	0.5056	37
33	1	3	0.3691	37
34	1	3	0.4202	38
35	1	3	0.6978	38
36	1	3	0.5398	42
37	1	3	0.3595	32
38	1	3	0.6221	29
39	1	3	0.2388	35
40	1	3	0.4413	35
41	1	3	0.5828	38
42	1	3	0.4380	35
43	1	3	0.3979	35
44	1	3	0.4759	42
45	1	3	0.6234	39
46	1	4	0.2031	32
47	1	4	0.5288	43
48	1	4	0.4441	28
49	1	4	0.4615	39
50	1	4	0.5092	42
51	1	4	0.3842	37
52	1	4	0.3911	36
53	1	4	0.4267	34
54	1	4	0.4446	38
55	1	4	0.2835	36

Obs.	CONC	REP	WEIGHT	LENGTH
56	1	4	0.6617	38
57	1	4	0.3288	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
73	2	1	0.6027	34
74	2	1	0.4826	28
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
80	2	2	0.5348	36
81	2	2	0.3229	31
82	2	2	0.6492	33
83	2	2	0.4800	34
84	2	2	0.4860	31
85	2	2	0.6186	41
86	2	2	0.2768	37
87	2	2	0.2293	40
88	2	2	0.4131	31
89	2	2	0.3322	34
90	2	2	0.3635	38
91	2	2	0.2916	42
92	2	2	0.7836	37
93	2	2	0.4338	30
94	2	2	0.3539	39
95	2	3	0.8085	43
96	2	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	29
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	0.3557	41
107	2	3	0.5946	29
108	2	3	0.7557	33
109	2	3	0.2234	37
110	2	4	0.4301	41

Obs.	CONC	REP	WEIGHT	LENGTH
111	2	4	0.6339	42
112	2	4	0.4964	41
113	2	4	0.6432	42
114	2	4	0.3927	39
115	2	4	0.4869	38
116	2	4	0.6060	41
117	2	4	0.7120	32
118	2	4	0.6233	32
119	2	4	0.4942	36
120	2	4	0.2892	35
121	2	4	0.5656	37
122	2	4	0.4410	39
123	2	4	0.7142	34
124	2	4	0.3220	40
125	2	4	0.5646	39
126	2	4	0.6874	40
127	2	4	0.4877	39
128	3	1	0.2708	41
129	3	1	0.1528	42
130	3	1	0.5708	36
131	3	1	0.7316	39
132	3	1	0.4342	32
133	3	1	0.4049	29
134	3	1	0.2140	38
135	3	1	0.2277	34
136	3	1	0.1866	42
137	3	1	0.4401	38
138	3	1	0.2975	33
139	3	1	0.4543	32
140	3	1	0.4439	28
141	3	1	0.5273	37
142	3	1	0.5392	38
143	3	1	0.2911	37
144	3	1	0.2973	37
145	3	1	0.3961	28
146	3	2	0.4092	33
147	3	2	0.3724	36
148	3	2	0.6042	41
149	3	2	0.3240	36
150	3	2	0.4016	29
151	3	2	0.6238	35
152	3	2	0.4107	36
153	3	2	0.2473	39
154	3	2	0.4104	34
155	3	2	0.4291	26
156	3	2	0.5000	36
157	3	2	0.5753	39
158	3	2	0.6219	38
159	3	2	0.5231	35
160	3	2	0.5368	37
161	3	2	0.2233	38
162	3	2	0.3719	41
163	3	3	0.3860	34
164	3	3	0.3609	34
165	3	3	0.3284	38

Obs.	CONC	REP	WEIGHT	LENGTH
166	3	3	0.3062	43
167	3	3	0.2936	36
168	3	3	0.5042	38
169	3	3	0.3304	36
170	3	3	0.5160	35
171	3	3	0.3472	34
172	3	3	0.4991	34
173	3	3	0.3032	34
174	3	3	0.4324	33
175	3	3	0.6812	32
176	3	3	0.3662	32
177	3	3	0.4562	32
178	3	3	0.4824	36
179	3	4	0.1818	38
180	3	4	0.5208	37
181	3	4	0.5037	36
182	3	4	0.3924	36
183	3	4	0.3928	37
184	3	4	0.3992	36
185	3	4	0.6094	36
186	3	4	0.4578	27
187	3	4	0.4852	37
188	3	4	0.5718	37
189	3	4	0.6113	34
190	3	4	0.4553	35
191	3	4	0.4939	39
192	3	4	0.5129	37
193	3	4	0.4252	38
194	3	4	0.2992	40
195	3	4	0.4283	36
196	4	1	0.5136	40
197	4	1	0.5024	34
198	4	1	0.4598	32
199	4	1	0.5026	35
200	4	1	0.4733	37
201	4	1	0.5604	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
206	4	1	0.4142	34
207	4	1	0.3394	39
208	4	1	0.3078	38
209	4	1	0.3045	39
210	4	1	0.4225	36
211	4	2	0.0746	37
212	4	2	0.5357	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
217	4	2	0.1773	36
218	4	2	0.4253	37
219	4	2	0.4570	38
220	4	2	0.5843	35

Obs.	CONC	REP	WEIGHT	LENGTH
221	4	2	0.4709	38
222	4	2	0.3806	40
223	4	2	0.5181	37
224	4	2	0.5576	22
225	4	2	0.3934	37
226	4	2	0.5770	37
227	4	2	0.5306	40
228	4	2	0.4299	38
229	4	2	0.4980	35
230	4	2	0.3225	40
231	4	3	0.4438	34
232	4	3	0.4452	35
233	4	3	0.5112	38
234	4	3	0.5531	31
235	4	3	0.6285	35
236	4	3	0.5685	38
237	4	3	0.4795	38
238	4	3	0.1753	34
239	4	3	0.3593	37
240	4	3	0.5806	40
241	4	3	0.2876	40
242	4	3	0.2649	32
243	4	3	0.5778	40
244	4	3	0.3560	41
245	4	3	0.3239	34
246	4	3	0.3079	32
247	4	3	0.3838	33
248	4	4	0.4114	28
249	4	4	0.5404	35
250	4	4	0.6109	33
251	4	4	0.4529	35
252	4	4	0.3917	40
253	4	4	0.3281	38
254	4	4	0.3417	35
255	4	4	0.8538	34
256	4	4	0.5791	31
257	4	4	0.2737	35
258	4	4	0.5295	37
259	4	4	0.4856	39
260	4	4	0.3665	38
261	4	4	0.2079	33
262	4	4	0.3784	34
263	4	4	0.4606	39
264	4	4	0.3634	38
265	4	4	0.4559	38
266	5	1	0.3762	38
267	5	1	0.3910	34
268	5	1	0.3514	34
269	5	1	0.5104	29
270	5	1	0.6587	38
271	5	1	0.5451	34
272	5	1	0.2212	37
273	5	1	0.3304	39
274	5	1	0.3457	34
275	5	1	0.4871	33

Obs.	CONC	REP	WEIGHT	LENGTH
276	5	1	0.3302	36
277	5	1	0.3410	35
278	5	1	0.3464	38
279	5	1	0.4366	35
280	5	1	0.4036	36
281	5	1	0.5985	34
282	5	1	0.3511	34
283	5	2	0.6452	32
284	5	2	0.1784	36
285	5	2	0.4350	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
290	5	2	0.5626	35
291	5	2	0.4098	38
292	5	2	0.4065	32
293	5	2	0.5261	35
294	5	2	0.4201	38
295	5	2	0.3822	35
296	5	2	0.6340	34
297	5	2	0.1940	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	5	3	0.4940	38
303	5	3	0.3694	33
304	5	3	0.3831	30
305	5	3	0.3492	37
306	5	3	0.4435	35
307	5	3	0.1982	42
308	5	3	0.3792	36
309	5	3	0.3379	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	5	4	0.4213	38
315	5	4	0.3665	33
316	5	4	0.4254	37
317	5	4	0.4962	36
318	5	4	0.5218	38
319	5	4	0.5505	38
320	5	4	0.3384	32
321	5	4	0.3735	33
322	5	4	0.4990	36
323	5	4	0.2858	36
324	5	4	0.5730	27
325	5	4	0.3612	37
326	5	4	0.3732	35
327	5	4	0.2206	39
328	5	4	0.3105	31
329	5	4	0.5311	39
330	5	4	0.5603	33

Obs.	CONC	REP	WEIGHT	LENGTH
331	6	1	0.3493	34
332	6	1	0.1330	36
333	6	1	0.2972	22
334	6	1	0.3376	32
335	6	1	0.3452	31
336	6	1	0.3508	32
337	6	1	0.3558	34
338	6	1	0.2725	33
339	6	1	0.3124	33
340	6	1	0.3938	30
341	6	1	0.4028	30
342	6	1	0.4276	34
343	6	2	0.4092	37
344	6	2	0.2556	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	29
351	6	2	0.3646	32
352	6	2	0.4946	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
357	6	2	0.1748	33
358	6	2	0.3572	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	6	3	0.4757	38
365	6	3	0.4422	32
366	6	3	0.3213	36
367	6	3	0.3762	39
368	6	3	0.3888	35
369	6	3	0.2996	33
370	6	3	0.3308	33
371	6	3	0.3680	35
372	6	3	0.3311	34
373	6	4	0.2137	31
374	6	4	0.2300	37
375	6	4	0.3798	38
376	6	4	0.3462	31
377	6	4	0.4166	28
378	6	4	0.3110	34
379	6	4	0.5331	31
380	6	4	0.4163	28
381	6	4	0.4403	30
382	6	4	0.2506	34
383	6	4	0.4098	28
384	6	4	0.2128	35
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	32
389	7	1	0.1012	20
390	7	1	0.1153	20
391	7	1	0.1790	23
392	7	1	0.1358	23
393	7	2	0.1518	25
394	7	3	0.1034	20
395	7	4	0.2570	22
396	7	4	0.0707	28