

US EPA ARCHIVE DOCUMENT

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DP Barcode : D183061  
PC Code No : 101101  
EEB Out :

FEB 17 1993

To: Walter Waldrop  
Product Manager 71  
Special Review and Reregistration Division (H7508W)

From: Anthony F. Maciorowski, Chief  
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 101101-  
Chemical Name : Metribuzin  
Type Product : Herbicide  
Product Name : Sencor  
Company Name : Miles, Inc.  
Purpose : Submission of fish early life-stage and aquatic invertebrate life-cycle studies under 6(a)(2) in support of reregistration.  
Action Code : 625 Date Due : 10/25/92  
Reviewer : H. Mansfield Date In : 10/01/92

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-						72-7(A)		
71-						72-7(B)		
71-						122-1(A)		
71-						122-1(B)		
71-						122-2		
71-						123-1(A)		
71-						123-1(B)		
71-						123-2		
71-				424478-01	S	124-1		
72-				424478-02	S	124-2		
72-						141-1		
72-						141-2		
72-...						141-5		

NOTE:  
Fish Early-life (424478-a)  
Up graded to COBE 1/11/94 (Key V.)

Y=Acceptable (Study satisfied Guideline)/Concur  
P=Partial (Study partially fulfilled Guideline but additional information is needed)  
S=Supplemental (Study provided useful information but Guideline was not satisfied)  
N=Unacceptable (Study was rejected)/Nonconcur



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Sencor--Data Evaluation & 6(a)2 Data  
Barcodes: D183061  
ID No: 101101

FROM: Anthony F. Maciorowski, Chief *Anthony F. Maciorowski*  
Ecological Effects Branch  
Environmental Fate and Effects Division (H7507C)

TO: Walter Waldrop, PM 71  
Reregistration Branch  
Special Review/Reregistration Division (H7508W)

**BACKGROUND**

Miles Inc. submitted two aquatic studies in support of the reregistration of metribuzen (sencor):

Guideline 72-4(a). Gagliano, G.G. and D.J. Roney. 1992. Early Life Stage Toxicity of SENCOR Technical to the Rainbow Trout *Oncorhynchus mykiss* Under Flow-Through Conditions. Miles Report No. 103249. Performed by Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 424478-01.

Guideline 72-4(b). Gagliano, G.G. and L.M. Bowers. 1992. Chronic Toxicity of SENCOR Technical to the Waterflea (*Daphnia magna*) Under Flow-Through Conditions. Miles Report No. 103248. Performed by Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 424478-02.



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**RESPONSE**

Neither of the aquatic organism studies satisfy the guideline requirements. Larval length in the trout study and reproduction in the daphnid study were significantly affected at all exposure levels. Therefore, an MATC could not be determined for either study. The daphnid study is currently being repeated, according to the registrant, and should soon be submitted. The trout study should also be repeated and a precise NOEC found for larval length.

These studies are also classified as 6(a)2 data. EEB will assess the chronic risk to aquatic organisms upon evaluation of the pending studies.

If you have any questions, please contact Heather Mansfield (305-5064).

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Metribuzen (SENCOR). Shaughnessey No. 101101.

2. **TEST MATERIAL:** SENCOR Technical [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one]; Batch No. 0145346/0030147; CAS No. 21087-64-9; 93% active ingredient; a white powder.

3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Toxicity Test. Species Tested: *Daphnia magna*

4. **CITATION:** Gagliano, G.G. and L.M. Bowers. 1992. Chronic Toxicity of SENCOR Technical to the Waterflea (*Daphnia magna*) Under Flow-Through Conditions. Miles Report No. 103248. Performed by Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 424478-02.

5. **REVIEWED BY:**

Heather N. Mansfield, Zoologist  
Ecological Effects Branch  
Environmental Fate and Effects Division

Signature: *Heather Mansfield*  
Date: 2/16/93

6. **APPROVED BY:**

Norman J. Cook, Head, Section 2  
Ecological Effects Branch  
Environmental Fate and Effects Division

Signature: *Norman J. Cook*  
Date: 12.17.93

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a freshwater invertebrate life-cycle toxicity test. Reproduction was significantly affected at all exposure levels when compared to the dilution water control data, therefore, the MATC for *Daphnia magna* exposed to SENCOR Technical could not be determined. The LOEC was 0.32 mg a.i./l mean measured concentration, the lowest concentration tested. The study author indicates that another study has been initiated as DMF apparently affected daphnid reproduction.

8. **RECOMMENDATIONS:** The study should be repeated to find the NOEC for reproduction and to determine the MATC.

9. **BACKGROUND:**

4

## DATA EVALUATION RECORD

1. **CHEMICAL:** Metribuzen (SENCOR). Shaughnessey No. 101101.
2. **TEST MATERIAL:** SENCOR Technical [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one]; Batch No. 0145346/0030147; CAS No. 21087-64-9; 93% active ingredient; a white powder.
3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** Gagliano, G.G. and L.M. Bowers. 1992. Chronic Toxicity of SENCOR Technical to the Waterflea (*Daphnia magna*) Under Flow-Through Conditions. Miles Report No. 103248. Performed by Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 424478-02.
5. **REVIEWED BY:**  
 Rosemary Graham Mora, M.S.  
 Associate Scientist  
 KBN Engineering and  
 Applied Sciences, Inc.  
 Signature: *Rosemary Graham Mora*  
 Date: 12/17/92
6. **APPROVED BY:**  
 Pim Kosalwat, Ph.D.  
 Senior Scientist  
 KBN Engineering and  
 Applied Sciences, Inc.  
 Signature: *P. Kosalwat*  
 Date: 12/17/92  
 Henry T. Craven, M.S.  
 Supervisor, EEB/EFED  
 USEPA  
 Signature: *Henry T. Craven*  
 Date: 2/16/93
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a freshwater invertebrate life-cycle toxicity test. Reproduction was significantly affected at all exposure levels when compared to the dilution water control data, therefore, the MATC for *Daphnia magna* exposed to SENCOR Technical could not be determined. The LOEC was 0.32 mg a.i./l mean measured concentration, the lowest concentration tested.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

A. Test Animals: *Daphnia magna* were obtained from 15-day old adult daphnids which were cultured in-house. The cultures were maintained under test conditions at  $20 \pm 1^\circ\text{C}$  under a photoperiod of 16 hours of light. The daphnids were fed a minimum of three times per week a combination of green algae (*Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*) and supplemented with a trout chow, yeast, and cereal leaf suspension.

B. Test System: The test system was a Mount-Brungs diluter which delivered test solutions to the test chambers. The test vessels were 1-l borosilicate glass beakers filled with 900 ml of test solution. The flow rate to each test vessel was 250 ml every 74 minutes resulting in five volume turnovers per day.

The test chambers were randomly positioned in a water bath. Sixteen hours of light at an intensity of 48-60 footcandles were provided each day. Thirty-minute dawn/dusk simulation periods were provided.

The dilution water was filtered spring water which was supplemented with treated (dechlorinated and filtered) city water. A representative sample of the dilution water had a hardness and alkalinity of 184 and 125 mg/l as  $\text{CaCO}_3$ , respectively; a pH range of 7.7-8.2; a specific conductivity of 356  $\mu\text{mhos/cm}$ ; and a residual chlorine of  $<0.003$  mg/l.

A primary stock solution (49,998 mg a.i./l) was prepared by dissolving 2.6881 g of test material in 50 ml of dimethylformamide (DMF).

C. Dosage: Twenty-one day, flow-through test. Based on results of preliminary testing, nominal test concentrations selected were 0.32, 0.63, 1.25, 2.5, and 5.0 mg a.i./l. A dilution water control and solvent control were also included. The solvent control had a DMF concentration of 99  $\mu\text{l/l}$ .

D. Design: Ten first-instar daphnids ( $<24$  hours old) were randomly distributed to each of four test vessels per treatment (i.e., 40 daphnids/treatment). The loading rate was 1 daphnid per 100 ml of test solution.

The daphnids were fed an equal amount per test vessel of an algal suspension (*Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*) at a rate of  $1 \times 10^7$  cells/l. A trout chow, yeast, and cereal leaf suspension was dispensed every third day at a rate of 0.5 ml/l.

Observations of survival, sublethal effects, and first brood of the organisms were recorded daily. After the release of first brood, observations of mortality and number of young produced were recorded every Monday, Wednesday, and Friday. At test termination, total body length of each surviving adult was recorded.

Dissolved oxygen concentration (DO), pH, conductivity, temperature, total hardness and alkalinity in alternating replicates of the controls, low, middle, and high concentrations were measured on days 1, 9, 16, and 20. Temperature was also monitored hourly in one centrally-located test chamber.

Samples of fresh test solutions were collected from alternating replicates (A and B or C and D) on test days -1, 0, 6, 13, and 20 for determination of SENCOR concentrations. Composite samples of the two replicates were analyzed using high pressure liquid chromatography.

- E. **Statistics:** Data for the replicate vessels for each concentration were grouped together for analysis. For this study, each parameter was tested for assumptions of normality (chi-square test), for homogeneity of variance (Bartlett's test), and to determine if control and solvent control data could be pooled (t-test).

Survival, time to first brood and adult length data were analyzed using Fisher's Exact test. A one-way analysis of variance (ANOVA) coupled with Dunnett's one-tailed multiple means comparison test was used to assess treatment effects on reproduction. Statistical conclusions were made at 95% confidence level.

12. **REPORTED RESULTS:** Mean measured concentrations were 0.32, 0.66, 1.32, 2.6, and 5.62 mg a.i./l (Table 2, attached). The measured concentrations ranged from 100 to 112% of nominal concentrations.

"Due to a separation between the silicone adhesive and the mesh covering the sidedrain, five daphnids in Solvent

Control Replicate [A] were washed down the drain. The problem was noted during Day 4 morning observations. The daphnids were transferred to a new test vessel and the study continued without further problems of this nature."

Adult survival in the exposure levels was not significantly different from that of the solvent control (Table 5, attached). Based on immobilization, the 21-day EC<sub>50</sub> was determined to be >5.62 mg a.i./l.

Length of the surviving adults was not significantly affected at any test level when compared to the solvent control data (Table 7, attached).

Time to first brood at 5.62 mg a.i./l was significantly reduced when compared to the pooled control data. The number of young produced per adult reproduction day after 21 days was significantly affected at the two highest exposure levels when compared to the solvent control (Table 6, attached). There was a significant difference in reproduction between the dilution water control and solvent control. The mean number of offspring per adult reproduction day of the dilution water control and solvent control was 7.43 and 4.64, respectively.

During the test, pH was 7.7-8.2; DO was 4.7-8.8 mg/l (52-97% of saturation at 20°C); temperature was 19.8-20.9°C; and hardness ranged from 150 to 172 mg/l.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**  
 "Based on the statistical analysis of survival, time to first brood, number of offspring per adult per reproduction day and parent daphnid length the no-observed-effect-concentration (NOEC) was 1.32 mg/l and the lowest-observed-effect-concentration (LOEC) was 2.60 mg/l. The point estimate MATC value was calculated to be 1.85 mg/l."

"Although there was clearly an effect of the test compound on daphnid reproduction in the high test levels, another study has been initiated due to the apparent solvent effect in this test. The report from the repeat study will be available in the near future."

Good laboratory practice (GLP) compliance and quality assurance statements were included in the report indicating that the study was performed in accordance with EPA GLP regulations (40 CFR Part 160).

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

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- A. **Test Procedure:** An SEP for a daphnid chronic flow-through test is not available at this time. Therefore, the ASTM guidelines were used in this data validation process. The test procedure follows the intent of the ASTM guidelines, except for the following deviations:

Based on the reviewer's results, reproduction was affected at all concentrations; therefore, no NOEC was determined.

The authors stated that water quality parameters (pH, DO, conductivity, hardness, and alkalinity) were recorded on days 1, 9, 16, and 20; however, alkalinity and conductivity measurements were not reported.

- B. **Statistical Analysis:** The reviewer used Toxstat (Version 3.1) computer program to compare the survival data and reproductive data (number of young/adult reproduction day) of the test concentrations to the dilution water control. Since the survival data failed the homogeneity test, a non-parametric test (Kruskal-Wallis) was used to analyze the data. This analysis demonstrated no significant difference between the control and any treatment levels (printouts, attached). The reproduction data met assumptions of homogeneity of variance and normality; therefore, Williams test was used to analyze the data. The results illustrated a significant difference at all exposure levels when compared to the dilution water control.

Individual length data were analyzed using a 2-way analysis of variance (ANOVA) coupled with Bonferroni's test for treatment comparisons (printout, attached). Length was significantly affected at the 4 lowest test concentrations when compared to the solvent control. No significant difference was noted at the highest test concentration. These results differ from those of the authors.

- C. **Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a freshwater invertebrate life-cycle toxicity test. Reproduction was affected at all exposure levels of SENCOR Technical when compared to the dilution water control data. The reviewer chose to compare the treatments to the dilution water control because the solvent in this test appeared to adversely affect the daphnid reproduction. Since the solvent was not present at the same concentration at all test levels (higher concentration with higher SENCOR level),

comparing the treatments to the solvent control may underestimate the toxicity of the test material. The MATC for *Daphnia magna* could not be determined. The LOEC was 0.32 mg a.i./l, the lowest concentration tested.

D. Adequacy of the Study:

- (1) Classification: Supplemental.
- (2) Rationale: No NOEC was determined.
- (3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, December 14, 1992.

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Metricubuzin

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

Pages 11 through 14 are not included.

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The material not included contains the following type of information:

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  - Identity of product impurities.
  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
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  - A draft product label.
  - The product confidential statement of formula.
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SENCOR Technical: Reproduction of Exposed Daphnia magna  
File: 42447802.rep Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

---

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	12	8	8	0

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Calculated Chi-Square goodness of fit test statistic = 8.6801  
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

SENCOR Technical: Reproduction of Exposed Daphnia magna  
File: 42447802.rep Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

---

Calculated B statistic = 15.66  
Table Chi-square value = 16.81 (alpha = 0.01)  
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00  
Used for Chi-square table value ==> df (#groups-1) = 6

---

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: SENCOR Technical: Reproduction of Exposed Daphnia magna  
 FILE: 42447802.rep  
 TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	8.1700	8.1700
1	Control	2	6.9200	6.9200
1	Control	3	6.9900	6.9900
1	Control	4	7.6400	7.6400
2	Solvent Control	1	7.5800	7.5800
2	Solvent Control	2	3.5800	3.5800
2	Solvent Control	3	3.8400	3.8400
2	Solvent Control	4	3.5700	3.5700
3	0.32 mg ai/l	1	4.3000	4.3000
3	0.32 mg ai/l	2	3.3300	3.3300
3	0.32 mg ai/l	3	3.4900	3.4900
3	0.32 mg ai/l	4	4.9400	4.9400
4	0.66 mg ai/l	1	2.7200	2.7200
4	0.66 mg ai/l	2	2.6300	2.6300
4	0.66 mg ai/l	3	4.6600	4.6600
4	0.66 mg ai/l	4	3.3400	3.3400
5	1.32 mg ai/l	1	3.2100	3.2100
5	1.32 mg ai/l	2	3.8000	3.8000
5	1.32 mg ai/l	3	2.1400	2.1400
5	1.32 mg ai/l	4	1.3300	1.3300
6	2.6 mg ai/l	1	1.0600	1.0600
6	2.6 mg ai/l	2	0.9200	0.9200
6	2.6 mg ai/l	3	0.9200	0.9200
6	2.6 mg ai/l	4	2.0200	2.0200
7	5.62 mg ai/l	1	0.3200	0.3200
7	5.62 mg ai/l	2	0.0500	0.0500
7	5.62 mg ai/l	3	0.1100	0.1100
7	5.62 mg ai/l	4	0.1000	0.1000

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	7.430	7.430	7.430
2	Solvent Control	4	4.643	4.643	4.643
3	0.32 mg ai/l	4	4.015	4.015	4.015
4	0.66 mg ai/l	4	3.338	3.338	3.338
5	1.32 mg ai/l	4	2.620	2.620	2.620
6	2.6 mg ai/l	4	1.230	1.230	1.230
7	5.62 mg ai/l	4	0.145	0.145	0.145

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	7.430				
Solvent Control	4.643	3.902	*	1.72	k= 1, v=21
0.32 mg ai/l	4.015	4.780	*	1.80	k= 2, v=21
0.66 mg ai/l	3.338	5.729	*	1.83	k= 3, v=21
1.32 mg ai/l	2.620	6.733	*	1.84	k= 4, v=21
2.6 mg ai/l	1.230	8.679	*	1.85	k= 5, v=21
5.62 mg ai/l	0.145	10.198	*	1.85	k= 6, v=21

s = 1.010

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

SENCOR Technical: Survival of Exposed Daphnia magna  
File: 42447802.sur Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

---

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	4	21	3	0

---

Calculated Chi-Square goodness of fit test statistic = 16.9199  
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

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

SENCOR Technical: Survival of Exposed Daphnia magna  
File: 42447802.sur Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

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D = 0.090

W = 0.723

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

---

Data FAIL normality test. Try another transformation.

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

SENCOR Technical: Survival of Exposed Daphnia magna  
File: 42447802.sur Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance  
Bartlett's test for homogeneity of variance

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These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.  
Additional transformations are useless.

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SENCOR Technical: Survival of Exposed Daphnia magna  
 File: 42447802.sur Transform: ARC SINE(SQUARE ROOT(Y))

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	1.219	0.875	24.000
2	Solvent Control	1.412	1.000	68.000
3	0.32 mg ai/l	1.412	1.000	68.000
4	0.66 mg ai/l	1.412	1.000	68.000
5	1.32 mg ai/l	1.412	1.000	68.000
6	2.6 mg ai/l	1.412	1.000	68.000
7	5.62 mg ai/l	1.331	0.950	42.000

Calculated H Value = 15.884 Critical H Value Table = 12.590  
 Since Calc H > Crit H REJECT Ho: All groups are equal.

SENCOR Technical: Survival of Exposed Daphnia magna  
 File: 42447802.sur Transform: ARC SINE(SQUARE ROOT(Y))

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP						
				0	0	0	0	0	0	0
1	Control	1.219	0.875	1	7	3	4	5	6	2
7	5.62 mg ai/l	1.331	0.950	.	.	.	.	.	.	.
3	0.32 mg ai/l	1.412	1.000	.	.	.	.	.	.	.
4	0.66 mg ai/l	1.412	1.000	.	.	.	.	.	.	.
5	1.32 mg ai/l	1.412	1.000	.	.	.	.	.	.	.
6	2.6 mg ai/l	1.412	1.000	.	.	.	.	.	.	.
2	Solvent Control	1.412	1.000	.	.	.	.	.	.	.

\* = significant difference (p=0.05)  
 Table q value (0.05,7) = 3.038

. = no significant difference  
 SE = 3.879

TITLE: SENCOR Technical: Survival of Exposed Daphnia magna  
 FILE: 42447802.sur  
 TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.4120
1	Control	2	0.8000	1.1071
1	Control	3	0.9000	1.2490
1	Control	4	0.8000	1.1071
2	Solvent Control	1	1.0000	1.4120
2	Solvent Control	2	1.0000	1.4120
2	Solvent Control	3	1.0000	1.4120
2	Solvent Control	4	1.0000	1.4120
3	0.32 mg ai/l	1	1.0000	1.4120
3	0.32 mg ai/l	2	1.0000	1.4120
3	0.32 mg ai/l	3	1.0000	1.4120
3	0.32 mg ai/l	4	1.0000	1.4120
4	0.66 mg ai/l	1	1.0000	1.4120
4	0.66 mg ai/l	2	1.0000	1.4120
4	0.66 mg ai/l	3	1.0000	1.4120
4	0.66 mg ai/l	4	1.0000	1.4120
5	1.32 mg ai/l	1	1.0000	1.4120
5	1.32 mg ai/l	2	1.0000	1.4120
5	1.32 mg ai/l	3	1.0000	1.4120
5	1.32 mg ai/l	4	1.0000	1.4120
6	2.6 mg ai/l	1	1.0000	1.4120
6	2.6 mg ai/l	2	1.0000	1.4120
6	2.6 mg ai/l	3	1.0000	1.4120
6	2.6 mg ai/l	4	1.0000	1.4120
7	5.62 mg ai/l	1	1.0000	1.4120
7	5.62 mg ai/l	2	1.0000	1.4120
7	5.62 mg ai/l	3	0.9000	1.2490
7	5.62 mg ai/l	4	0.9000	1.2490

SENCOR : Daphnia magna

ANOVA on Lengths

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT	1.0000	2.0000	3.0000	4.0000	5.0000	6.0000
	7.0000					
REP	1.0000	2.0000	3.0000	4.0000		

---

DEP VAR: LENGTH N: 268 MULTIPLE R: 0.784 SQUARED MULTIPLE R: 0.614

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	2.2779	6	0.3797	22.3754	0.0000
REP	0.9135	3	0.3045	17.9467	0.0000
TRT*REP	3.3718	18	0.1873	11.0402	0.0000
ERROR	4.0722	240	0.0170		

---

Post-hoc pairwise comparison of length/Bonferroni.

COL/

ROW	TRT
1	1.0000
2	2.0000
3	3.0000
4	4.0000
5	5.0000
6	6.0000
7	7.0000

USING LEAST SQUARES MEANS.

POST HOC TEST OF LENGTH

MATRIX OF PAIRWISE MEAN DIFFERENCES:

	1	2	3	4	5
1	0.0000				
2	0.2476	0.0000			
3	-0.0224	-0.2700	0.0000		
4	-0.0024	-0.2500	0.0200	0.0000	
5	0.0901	-0.1575	0.1125	0.0925	0.0000
6	0.1151	-0.1325	0.1375	0.1175	0.0250
7	0.1848	-0.0628	0.2072	0.1872	0.0947

	6	7
6	0.0000	
7	0.0697	0.0000

BONFERRONI ADJUSTMENT.

MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	0.0000	1.0000			
3	1.0000	0.0000	1.0000		
4	1.0000	0.0000	1.0000	1.0000	
5	0.0666	0.0000	0.0030	0.0355	1.0000
6	0.0037	0.0005	0.0001	0.0015	1.0000
7	0.0000	0.9623	0.0000	0.0000	0.0319

	6	7
6	1.0000	
7	0.3994	1.0000

→ }  
greater than control

1 = dilution water control  
2 = solvent control

SENCOR : Daphnia magna

THE FOLLOWING RESULTS ARE FOR:  
TRT = 1.0000

TOTAL OBSERVATIONS: 35

LENGTH

N OF CASES	35
MINIMUM	4.1000
MAXIMUM	4.9000
MEAN	4.6057
STANDARD DEV	0.1697

THE FOLLOWING RESULTS ARE FOR:  
TRT = 2.0000

TOTAL OBSERVATIONS: 35

LENGTH

N OF CASES	35
MINIMUM	4.3000
MAXIMUM	5.1000
MEAN	4.8314
STANDARD DEV	0.1641

THE FOLLOWING RESULTS ARE FOR:  
TRT = 3.0000

TOTAL OBSERVATIONS: 40

LENGTH

N OF CASES	40
MINIMUM	4.2000
MAXIMUM	5.0000
MEAN	4.5775
STANDARD DEV	0.1641

THE FOLLOWING RESULTS ARE FOR:  
TRT = 4.0000

TOTAL OBSERVATIONS: 40

LENGTH

N OF CASES	40
MINIMUM	4.1000
MAXIMUM	5.0000
MEAN	4.5975
STANDARD DEV	0.2412

THE FOLLOWING RESULTS ARE FOR:  
TRT = 5.0000

TOTAL OBSERVATIONS: 40

LENGTH

N OF CASES 40  
MINIMUM 4.3000  
MAXIMUM 5.0000  
MEAN 4.6900  
STANDARD DEV 0.1722

THE FOLLOWING RESULTS ARE FOR:  
TRT = 6.0000

TOTAL OBSERVATIONS: 40

LENGTH

N OF CASES 40  
MINIMUM 4.2000  
MAXIMUM 5.0000  
MEAN 4.7150  
STANDARD DEV 0.1942

THE FOLLOWING RESULTS ARE FOR:  
TRT = 7.0000

TOTAL OBSERVATIONS: 38

LENGTH

N OF CASES 38  
MINIMUM 4.4000  
MAXIMUM 5.0000  
MEAN 4.7816  
STANDARD DEV 0.1249

---

SUMMARY STATISTICS FOR LENGTH

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 17.9172 DF= 6 PROBABILITY = 0.0064

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	2.1299	6	0.3550	11.0062	0.0000
WITHIN GROUPS	8.4179	261	0.0323		

---

KOLMOGOROV-SMIRNOV ONE SAMPLE TEST USING STANDARD NORMAL DISTRIBUTION

VARIABLE	N-OF-CASES	MAXDIF	PROBABILITY (2-TAIL)
LENGTH	268.0000	1.0000	0.0000

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Metribuzen (SENCOR). Shaughnessey No. 101101.
- 2. **TEST MATERIAL:** SENCOR Technical [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one]; Batch No. 1145430/2030001; CAS No. 21087-64-9; 94% active ingredient; an off-white powder.
- 3. **STUDY TYPE:** 72-4. Freshwater Fish Early Life-Stage Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*)
- 4. **CITATION:** Gagliano, G.G. and D.J. Roney. 1992. Early Life Stage Toxicity of SENCOR Technical to the Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Miles Report No. 103249. Performed by Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 424478-01.

5. **REVIEWED BY:**

Heather N. Mansfield, Zoologist  
Ecological Effects Branch  
Environmental Fate and Effects Division

Signature: *Heather Mansfield*  
Date: 2/16/93

6. **APPROVED BY:**

Norman J. Cook, Head, Section 2  
Ecological Effects Branch  
Environmental Fate and Effects Division

Signature: *Norman Cook*  
Date: 02-17-93

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a fish early life-stage test. Larval length was significantly affected at all exposure levels, therefore, the MATC for *Oncorhynchus mykiss* exposed to SENCOR Technical could not be determined. The LOEC was 3.0 mg a.i./l, the lowest concentration tested.

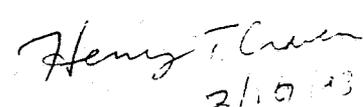
*Upgraded to CORE 2/11/94 [Signature]*

8. **RECOMMENDATIONS:** The study should be repeated in order to find an NOEC for larval length and the MATC.

9. **BACKGROUND:**

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

## DATA EVALUATION RECORD

1. **CHEMICAL:** Metribuzen (SENCOR). Shaughnessey No. 101101.
2. **TEST MATERIAL:** SENCOR Technical [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one]; Batch No. 1145430/2030001; CAS No. 21087-64-9; 94% active ingredient; an off-white powder.
3. **STUDY TYPE:** 72-4. Freshwater Fish Early Life-Stage Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).
4. **CITATION:** Gagliano, G.G. and D.J. Roney. 1992. Early Life Stage Toxicity of SENCOR Technical to the Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Miles Report No. 103249. Performed by Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 424478-01.
5. **REVIEWED BY:**  
 Rosemary Graham Mora, M.S.  
 Associate Scientist  
 KBN Engineering and  
 Applied Sciences, Inc.  
 Signature:   
 Date: 12/17/92  
 16-25
6. **APPROVED BY:**  
 Pim Kosalwat, Ph.D.  
 Senior Scientist  
 KBN Engineering and  
 Applied Sciences, Inc.  
 Signature: P. Kosalwat  
 Date: 12/17/92  
 Henry T. Craven, M.S.  
 Supervisor, EEB/EFED  
 USEPA  
 Signature:   
 Date: 2/15/93
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a fish early life-stage test. Larval length was significantly affected at all exposure levels, therefore, the MATC for *Oncorhynchus mykiss* exposed to SENCOR Technical could not be determined. The LOEC was 3.0 mg a.i./l, the lowest concentration tested.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

**11. MATERIALS AND METHODS:**

A. **Test Animals:** Unfertilized rainbow trout (*Oncorhynchus mykiss*) eggs and sperm which were collected from 3 adult females and 6 adult males were obtained from Mount Lassen Trout Farm, Red Bluff, California. The eggs and sperm were acclimated from 7°C to 10°C within 31 minutes. The eggs were placed in a dry container and the sperm were mixed with the eggs. Dilution water was added to the bowl and the egg-sperm mixture was gently stirred. The contents were allowed to stand for 1 minute after mixing. The eggs were rinsed with dilution water and allowed to harden for 2.25 hours.

B. **Test System:** The test system was a 2-1 proportional diluter which intermittently delivered test solutions to the test chambers. Each glass test aquarium (23 x 35 cm) had a solution depth of 25 cm resulting in a solution volume of 20 l. Embryo incubation cups were glass jars (9-cm diameter) with 40-mesh stainless steel screen bottoms. A rocker arm apparatus was used to gently oscillate incubation cups in the test chamber.

The test chambers were positioned in a water bath maintained at 10 ±2°C. Sixteen hours of light at an intensity of 50-70 footcandles were provided each day. Thirty-minute dawn/dusk simulation periods were provided.

The dilution water was filtered spring water which was supplemented with treated (dechlorinated and filtered) city water. A representative sample of the dilution water had a hardness and alkalinity of 51.6 and 44.8 mg/l as CaCO<sub>3</sub>, respectively, a pH range of 7.4-7.9, a specific conductivity of 118.1 μmhos/cm, and a residual chlorine of <0.003 mg/l.

A primary stock solution (97.025 g a.i./l) was prepared by dissolving 103.218 g of test material in 1 l of dimethylformamide (DMF).

C. **Dosage:** Ninety-five-day, flow-through test. Based on results of preliminary testing, nominal test concentrations selected were 3.2, 6.3, 13, 25, and 50 mg a.i./l. A dilution water control and two solvent controls were also included. Solvent control #1 and solvent control #2 had a DMF concentration of 0.5 and 0.1 ml/l, respectively.

- D. **Design:** Thirty-five embryos were impartially selected and distributed to each incubation cup. One cup was suspended in each of four replicate test chambers per treatment and control. Dead embryos were counted and removed daily.

For viability determination, 50 eggs were placed in extra incubation cups which were then suspended in each of the four control exposure chambers. After 11 days, egg viability was determined by clearing the eggs in a 10% acetic acid solution. The embryos were observed for evidence of embryonic development. The number of eggs hatched was recorded daily until hatch was complete on day 31 when 95% of the eggs in the controls had hatched.

On test day 34 (3 days post-hatch), the larvae were impartially reduced to 15 individuals per incubation cup. On test day 39, the larvae were released from the cups into their respective chambers. Beginning on test day 42 (11 days post-hatch), the larvae were fed live brine shrimp (*Artemia salina*) nauplii *ad libitum* two times daily. Ground trout/salmon starter was added to the diet on test day 48. The aquaria were siphoned when necessary.

Behavior, appearance, and mortality of larvae were observed and recorded daily. Dead individuals were removed when observed. On test day 67, the larvae were photographed in the test chambers with a scale on the bottom of the chamber. Larval length was recorded by projecting the photographic slides on a wall and measuring the fish with the photographed scale. At test termination, the larvae were counted, and individually weighed (wet, blotted dry) and measured.

Dissolved oxygen concentration (DO), pH, conductivity, temperature, total hardness and alkalinity in alternating replicates of the controls, low, middle, and high concentrations were measured on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, and 95. Temperature was monitored hourly in one centrally-located test chamber.

Samples of fresh test solutions were collected from alternating replicates on test days -1, 0, and at least weekly thereafter for determination of SENCOR concentrations. Samples were analyzed using high pressure liquid chromatography.

E. **Statistics:** The percentage survival and egg hatchability data were transformed (arcsine square-root percentage) before analysis. The data for the replicate chambers for each concentration were grouped together for analysis. For this study, each parameter was tested for normality (chi-square test), for homogeneity of variance (Bartlett's test), and to determine if control and solvent control data could be pooled (t-test). "The two highest test concentrations had levels of 0.5 and 0.25 ml/l; and three lower treatments had solvent levels approximating 0.1 ml/l. Given this, solvent controls were established at 0.5 ml/l and 0.1 ml/l. For statistical analysis, the 0.5 ml/l solvent control was considered for the highest two treatments and the 0.1 ml/l control for the three lower treatments."

Survival and egg hatchability data were analyzed using Fisher's Exact test. Percentage swim-up data were analyzed using Kruskal-Wallis ANOVA and Dunn's multiple comparison test. Analysis of variance (ANOVA) coupled with Dunnett's test were used to assess treatment effects on growth. Treatment levels that caused significant survival effects were excluded from the analysis of growth data. Statistical conclusions were made at 95% confidence level.

12. **REPORTED RESULTS:** Mean measured concentrations were 3.0, 5.7, 11.7, 23.5, and 48 mg a.i./l (Table 2, attached). The measured concentrations ranged from 90 to 96% of nominal concentrations.

Mean embryo viability in the control was 74%. Time to hatch was not significantly affected when compared to pooled control data. A significant difference in percent hatch was observed at 3.0 mg a.i./l when compared to the pooled control; this difference was considered a result of biological variability and not compound related (Table 6, attached). Time to 100% swim-up was affected at the highest test concentration when compared to the pooled control data. Sublethal affects were noted primarily in the highest test concentration. Survival of larvae at day 36 post-hatch and test termination was affected at the two highest test concentrations (23.5 and 48 mg a.i./l) when compared to the pooled control data (Table 6, attached).

"Due to a camera malfunction, photographs were not available for three replicates from the 3.0 test level. Since there was only one replicate from the 3.0 test level, the replicate variability within treatment (which was used as

the error term) was based on the variability of the eight replicates within the control group and the four replicates within the 5.7 and 11.7 mg/l test levels...The results of the statistical analysis showed that there was a significant difference in length between the pooled controls and the 5.7 and 11.7 mg/l test levels." Larval length at test termination was significantly reduced at 3.0 and 11.7 mg a.i./l when compared to the pooled control data. Wet weight showed no significant difference at 3.0, 5.7, and 11.7 mg a.i./l when compared to the solvent control #2 data.

During the study, the pH range was 7.3-7.9; total hardness and alkalinity ranges were 38-60 and 38-55 mg/l as CaCO<sub>3</sub>, respectively; the daily temperature measurements ranged from 10.1 to 13.8°C; conductivity ranged from 114 to 138 µmhos/cm; and DO ranged from 8.5 to 11.6 mg/l. During the first 68 days of the test, hourly temperature readings ranged from 10.5 to 12.7°C. The hourly monitoring was discontinued after day 68 due to the data logger malfunction.

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

For weight and survival at day 67 and day 95, the NOEC and LOEC were 11.7 and 23.5 mg a.i./l, respectively. For egg hatch and time to hatch, the NOEC was >48.0 mg a.i./l. The NOEC and LOEC for time to 100% swim-up were 23.5 and 48.0 mg a.i./l, respectively. The NOEC for length was <3.0 mg a.i./l.

Good laboratory practice (GLP) compliance and quality assurance statements were included in the report indicating that the study was performed in accordance with EPA GLP regulations (40 CFR Part 160).

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

A. **Test Procedure:** The test procedure is generally in accordance with the SEP, except for the following deviations:

Since larval length at test termination was reduced at all exposure levels, no NOEC could be determined. One concentration must adversely affect a life stage and one concentration must not adversely affect any life stage.

The test temperature ranged from 10.1 to 13.8°C; the SEP recommends 10 ±2°C during the exposure period.

The flow rate of test solution to the test vessels was not reported; the SEP recommends a flow rate which provides 90% replacement in 8-12 hours.

The report did not indicate whether food was withheld from the fish for at least 24 hours prior to test termination as recommended.

- B. **Statistical Analysis:** Individual length and weight data were analyzed using a 2-way analysis of variance (ANOVA) coupled with Bonferroni's test for treatment comparisons (printout, attached). The reviewer did not verify the authors' results for length and survival data collected on day 67 due to missing data.

Survival and hatchability data were transformed (arcsine square-root) and analyzed using Toxstat® (Version 3.3). The survival data failed to meet the assumptions of homogeneity of variance (Bartlett's or Hartley tests) and normality (Chi-square or Shapiro Wilks tests), therefore a non-parametric test (Kruskal-Wallis) was used to evaluate treatment effects (printouts, attached). The hatchability data met the assumptions of homogeneity of variance and normality, therefore, Williams test was used to analyze the data. The reviewer's analysis of survival data showed a significant reduction at the highest test concentration when compared to the controls. No significant reduction in hatchability was noted at any test concentration when compared to the control.

Length was significantly reduced at all test concentrations and weight was significantly reduced at 11.7 and 23.5 mg a.i./l (printouts, attached). Concentrations 11.7 and 23.5 mg a.i./l were compared to Solvent Control #1 and concentrations 3.0 and 5.7 mg a.i./l were compared to Solvent Control #2.

- C. **Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a fish early life-stage test. Larval length was affected at all exposure levels of SENCOR Technical, therefore, the MATC for rainbow trout could not be determined. The LOEC was 3.0 mg a.i./l, the lowest concentration tested.
- D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) Rationale: No NOEC was determined.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, December 9, 1992.

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metribuzin

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Page      is not included in this copy.

Pages 36 through 38 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.

---

Metribuzen: Survival of Exposed <sup>RBT</sup> Larvae at Test Term.  
File: C:\TOXSTAT\42447801.SUR Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

---

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.144	7.744	12.224	7.744	2.144
OBSERVED	0	4	23	5	0

---

Calculated Chi-Square goodness of fit test statistic = 16.5699  
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

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

Metribuzen: Survival of Exposed <sup>RBT</sup> Larvae at test term.  
File: C:\TOXSTAT\42447801.SUR Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

---

D = 0.075

W = 0.891

Critical W (P = 0.05) (n = 32) = 0.930

Critical W (P = 0.01) (n = 32) = 0.904

---

Data FAIL normality test. Try another transformation.

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

Metribuzen: Survival of Exposed <sup>CBT</sup> ~~EM~~ Larvae <sup>at test term</sup>  
File: C:\TOXSTAT\42447801.SUR Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance  
Bartlett's test for homogeneity of variance

---

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.  
Additional transformations are useless.

---

TITLE: Metribuzen: Survival of Exposed <sup>RBT</sup> ~~PH~~ Larvae at test term.  
 FILE: C:\TOXSTAT\42447801.SUR  
 TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.4413
1	Control	2	1.0000	1.4413
1	Control	3	1.0000	1.4413
1	Control	4	1.0000	1.4413
2	Solvent #1	1	1.0000	1.4413
2	Solvent #1	2	1.0000	1.4413
2	Solvent #1	3	1.0000	1.4413
2	Solvent #1	4	1.0000	1.4413
3	Solvent #2	1	1.0000	1.4413
3	Solvent #2	2	1.0000	1.4413
3	Solvent #2	3	1.0000	1.4413
3	Solvent #2	4	1.0000	1.4413
4	3.0 mg ai/l	1	0.9300	1.3030
4	3.0 mg ai/l	2	0.9300	1.3030
4	3.0 mg ai/l	3	1.0000	1.4413
4	3.0 mg ai/l	4	1.0000	1.4413
5	5.7 mg ai/l	1	1.0000	1.4413
5	5.7 mg ai/l	2	1.0000	1.4413
5	5.7 mg ai/l	3	1.0000	1.4413
5	5.7 mg ai/l	4	1.0000	1.4413
6	11.7 mg ai/l	1	1.0000	1.4413
6	11.7 mg ai/l	2	1.0000	1.4413
6	11.7 mg ai/l	3	1.0000	1.4413
6	11.7 mg ai/l	4	0.9300	1.3030
7	23.5 mg ai/l	1	0.6000	0.8861
7	23.5 mg ai/l	2	0.6000	0.8861
7	23.5 mg ai/l	3	0.5300	0.8154
7	23.5 mg ai/l	4	0.4000	0.6847
8	48 mg ai/l	1	0.0000	0.1295
8	48 mg ai/l	2	0.0000	0.1295
8	48 mg ai/l	3	0.0700	0.2678
8	48 mg ai/l	4	0.0000	0.1295

Metribuzen: Survival of Exposed <sup>RBT</sup> Larvae at test termin.  
 File: C:\TOXSTAT\42447801.SUR Transform: ARC SINE(SQUARE ROOT(Y))

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	1.441	1.000	88.000
2	Solvent #1	1.441	1.000	88.000
3	Solvent #2	1.441	1.000	88.000
4	3.0 mg ai/l	1.372	0.965	64.000
5	5.7 mg ai/l	1.441	1.000	88.000
6	11.7 mg ai/l	1.407	0.983	76.000
7	23.5 mg ai/l	0.818	0.533	26.000
8	48 mg ai/l	0.164	0.018	10.000

Calculated H Value = 26.882 Critical H Value Table = 14.070  
 Since Calc H > Crit H REJECT Ho: All groups are equal.

Metribuzen: Survival of Exposed <sup>RBT</sup> Larvae  
 File: C:\TOXSTAT\42447801.SUR Transform: ARC SINE(SQUARE ROOT(Y))

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP															
				0	0	0	0	0	0	0	0								
8	48 mg ai/l	0.164	0.018	\															
7	23.5 mg ai/l	0.818	0.533	.	\														
4	3.0 mg ai/l	1.372	0.965	.	.	\													
6	11.7 mg ai/l	1.407	0.983	.	.	.	\												
5	5.7 mg ai/l	1.441	1.000	*	.	.	.	\											
2	Solvent #1	1.441	1.000	*	.	.	.	.	\										
3	Solvent #2	1.441	1.000	*	.	.	.	.	.	\									
1	Control	1.441	1.000	*	.	.	.	.	.	.	\								

\* = significant difference (p=0.05)  
 Table q value (0.05,8) = 3.124

. = no significant difference  
 SE = 5.613

12BT

Metribuzen: Hatchability of Exposed ~~EM~~ Embryos  
File: 42447801.hat Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

---

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.144	7.744	12.224	7.744	2.144
OBSERVED	0	8	14	10	0

---

Calculated Chi-Square goodness of fit test statistic = 5.2117  
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Metribuzen: Hatchability of Exposed <sup>CBT</sup> Embryos  
File: 42447801.hat Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

---

D = 0.262

W = 0.965

Critical W (P = 0.05) (n = 32) = 0.930

Critical W (P = 0.01) (n = 32) = 0.904

---

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

45

Metribuzen: Hatchability of Exposed <sup>LOT</sup> Embryos  
File: 42447801.hat Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance

---

Calculated H statistic (max Var/min Var) = 16.40  
Closest, conservative, Table H statistic = 249.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) =	8,	df (# reps-1) =	3
Actual values ==>	R (# groups) =	8,	df (# avg reps-1) =	3.00

---

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Metribuzen: Hatchability of Exposed <sup>UBT</sup> ~~FHM~~ Embryos  
File: 42447801.hat Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

---

Calculated B statistic = 7.56  
Table Chi-square value = 18.48 (alpha = 0.01)  
Table Chi-square value = 14.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00  
Used for Chi-square table value ==> df (#groups-1) = 7

---

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: Metribuzen: Hatchability of Exposed <sup>RBT</sup> Embryos  
FILE: 42447801.hat  
TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.6600	0.9483
1	Control	2	0.5700	0.8556
1	Control	3	0.6300	0.9169
1	Control	4	0.7100	1.0021
2	Solvent #1	1	0.6900	0.9803
2	Solvent #1	2	0.7100	1.0021
2	Solvent #1	3	0.7400	1.0357
2	Solvent #1	4	0.5700	0.8556
3	Solvent #2	1	0.5700	0.8556
3	Solvent #2	2	0.6300	0.9169
3	Solvent #2	3	0.6600	0.9483
3	Solvent #2	4	0.8000	1.1071
4	3.0 mg ai/l	1	0.4600	0.7454
4	3.0 mg ai/l	2	0.5400	0.8254
4	3.0 mg ai/l	3	0.5700	0.8556
4	3.0 mg ai/l	4	0.5400	0.8254
5	5.7 mg ai/l	1	0.7100	1.0021
5	5.7 mg ai/l	2	0.6600	0.9483
5	5.7 mg ai/l	3	0.6900	0.9803
5	5.7 mg ai/l	4	0.5700	0.8556
6	11.7 mg ai/l	1	0.4600	0.7454
6	11.7 mg ai/l	2	0.6600	0.9483
6	11.7 mg ai/l	3	0.6900	0.9803
6	11.7 mg ai/l	4	0.6000	0.8861
7	23.5 mg ai/l	1	0.4600	0.7454
7	23.5 mg ai/l	2	0.5700	0.8556
7	23.5 mg ai/l	3	0.5700	0.8556
7	23.5 mg ai/l	4	0.8600	1.1873
8	48 mg ai/l	1	0.7400	1.0357
8	48 mg ai/l	2	0.6300	0.9169
8	48 mg ai/l	3	0.4900	0.7754
8	48 mg ai/l	4	0.5700	0.8556

RBT

Metribuzen: Hatchability of Exposed ~~FHM~~ Embryos  
File: 42447801.hat Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	0.642	0.931	0.952
2	Solvent #1	4	0.677	0.968	0.952
3	Solvent #2	4	0.665	0.957	0.952
4	3.0 mg ai/l	4	0.528	0.813	0.891
5	5.7 mg ai/l	4	0.658	0.947	0.891
6	11.7 mg ai/l	4	0.603	0.890	0.891
7	23.5 mg ai/l	4	0.615	0.911	0.891
8	48 mg ai/l	4	0.608	0.896	0.891

RBT

Metribuzen: Hatchability of Exposed ~~FHM~~ Embryos  
File: 42447801.hat Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.952				
Solvent #1	0.952	0.289		1.71	k= 1, v=24
Solvent #2	0.952	0.289		1.79	k= 2, v=24
3.0 mg ai/l	0.891	0.534		1.82	k= 3, v=24
5.7 mg ai/l	0.891	0.534		1.83	k= 4, v=24
11.7 mg ai/l	0.891	0.534		1.84	k= 5, v=24
23.5 mg ai/l	0.891	0.534		1.84	k= 6, v=24
48 mg ai/l	0.891	0.534		1.85	k= 7, v=24

s = 0.104

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