

5-17-89

Accession No. 407009-14

DATA EVALUATION RECORD

1. **CHEMICAL:** Ethyltrianol.  
Shaughnessey No. 128997.
2. **TEST MATERIAL:** HWG 1608 Technical, Lot #86R0082I, a tan powder, 96.3% purity.
3. **STUDY TYPE:** Freshwater Fish Early Life Stage Test.  
Species Tested: Rainbow Trout  
(Salmo gairdneri).
4. **CITATION:** Surprenant, D. C. 1988. The Toxicity of HWG 1608 Technical to Rainbow Trout (Salmo gairdneri) Embryos and Larvae. Report No. 87-11-2545. Conducted by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Mobay Corporation, Stilwell, Kansas. EPA Accession No. 407009-14.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.  
Staff Toxicologist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: P. Kosalwat  
Date: 4/3/89

6. **APPROVED BY:**

Isabel C. Johnson, M.S.  
Principal Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: Isabel C. Johnson  
Date: April 5, 1989

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

Signature:

Date:

5/17/89  
Henry T. Craven  
5/17/89

7. **CONCLUSIONS:** This study is scientifically sound. However, it does ~~not~~ fulfill the guideline requirements for a fish early life stage test since statistical analysis on growth data could not be verified. Embryo viability and survival of organisms at hatch were not affected by the concentrations of HWG Technical 1608 tested. Based on larval survival, the MATC value of HWG 1608 Technical for Salmo gairdneri was determined to be between 12 and 25 ug/L mean measured concentrations.

Core  
12/20/89

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



9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Unfertilized rainbow trout eggs and sperm were obtained from Mount Lassen Trout Farm, Red Bluffs, CA. Upon receipt, eggs and sperm were mixed in a stainless steel bowl with a small amount of dilution water. The embryos were allowed to remain undisturbed during the water-hardening period for approximately 45 minutes before using.

B. Test System: A modified constant flow serial diluter, with a 0.50 dilution factor, was used to prepare and deliver five nominal concentrations of HWG 1608 Technical, a dilution water control, and a solvent control (acetone concentration of 100 ul/L) to duplicate test aquaria.

The dilution and control water source was well water which was pumped into an epoxy-coated concrete reservoir where it was supplemented with Town of Wareham untreated well water and aerated. Weekly characterization of the well water established the total hardness and alkalinity ranges of 26-36 and 27-33 mg/L as  $\text{CaCO}_3$ , respectively; the pH range of 7.0-7.1; and the specific conductivity range of 100-130 umhos/cm during the study period.

Each glass test aquarium measured 39 x 20 x 25 cm with a 19.5-cm high side drain that maintained a constant exposure solution volume of 15 L. The diluter system continually delivered the control and test solutions to the exposure aquaria at a rate equivalent to provide approximately 6.5 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 8 hours. Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 16-mesh Nitex screen bottoms. A rocker arm apparatus was used to gently oscillate the incubation cups in the exposure solutions. Sixteen hours of light ranging from 30 to 70 footcandles at the water surface were provided each day. The aquaria were impartially positioned in a water bath containing circulating water cooled by a Frigid Unit<sup>R</sup> chiller designed to maintain the test solution temperatures at  $12 \pm 2^\circ\text{C}$ .



- C. Dosage: Eighty-three-day embryo-larval flow-through chronic test.
- D. Design: Based on static and flow-through, range-finding acute tests, the nominal concentrations of HWG 1608 Technical selected for the definitive early life stage exposure were 15, 30, 60, 120, and 240 ug/L. Fertilized eggs were impartially selected and distributed to each of 28 embryo incubation cups (50 eggs per cup, 4 cups per treatment level or control). After all the eggs were counted, two egg cups were suspended in each duplicate test aquarium.

Due to their extreme sensitivity to physical trauma during the early developmental stage, dead eggs within the egg cups were not removed between days 2 and 15 of the exposure period. After day 15, dead eggs were removed and preserved to determine the embryonic development. A definitive determination of viability was made on day 15, when all the eggs exhibited well-pronounced embryonic development. Any egg exhibiting embryonic development (up to and including day 15), whether dead or alive, was considered fertile for purposes of determining percent viability (i.e., number of viable embryos/numbers of eggs exposed). All non-viable eggs were discarded.

On test day 15, twenty live and viable embryos from each replicate treatment and control were impartially selected and placed into an incubation cup which was suspended in the respective exposure aquaria. The larvae which hatched from these isolated embryos were used to initiate the swim-up stage of the study. Hatching was deemed complete (test day 23) when no more than 5 unhatched viable embryos remained in any embryo incubation cup. Calculations of percentage survival of organisms at hatch were based on the number of live larvae per incubation cup after hatching was complete compared to the number of viable embryos.

To initiate the 60-day post-hatch larval exposure, the surviving larvae from each of the isolated embryo incubation cups within each replicate aquarium were released into their respective aquaria, providing a total of 20 larvae per replicate aquarium. Larvae were fed live brine shrimp (Artemia salina) nauplii three times daily on weekdays and twice daily on weekends and holidays when the rainbow trout reached swim-up stage



and began actively feeding (approximately 13 days post-hatch). Aquaria were brushed and siphoned when necessary (generally twice weekly) to remove excess food and fecal matter.

Behavior and appearance of larvae were observed daily and the number of surviving larvae was counted twice weekly. At 60 days post-hatch exposure (test termination), the percentage larval survival was determined. The larvae were measured and weighed individually to calculate the mean and standard deviation of total length and wet weight.

Dissolved oxygen concentration, pH and temperature were measured in each replicate aquarium on day 0 and subsequently measured daily in one replicate aquarium of each treatment level and the controls such that each aquarium was measured on alternating days. In addition, the temperature of the water bath surrounding the exposure aquaria was continually monitored throughout the study period. Total hardness as  $\text{CaCO}_3$  was measured on day 0 and weekly thereafter in alternating replicates of the high and low test concentrations and the controls. Samples were collected from all replicate test solutions and the controls on test days 1, 8, 14, 20, 28, and weekly thereafter until test termination and analyzed for HWG 1608 Technical.

- E. **Statistics:** Statistical analyses were performed using the mean organism response of each replicate aquarium. All statistical conclusions were made at 95% level of certainty except in the case of the Chi-Square Goodness of Fit test and the Bartlett's test, in which 99% level of certainty was applied.

One-way, single classification analyses of variance were conducted on each endpoint to compare with the control and solvent control data. These comparisons indicated that the presence of acetone in the exposure solutions did not affect viability, survival or growth of the test organisms. Consequently, the control and solvent control data were pooled for subsequent statistical analyses.

Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. For each endpoint, the performance of organisms at each dose level of HWG 1608 Technical was compared with that of



the pooled controls using Williams' Test. Larval survival data were analyzed before larval length and weight. Dose levels that caused significant survival effects were excluded from the analyses of larval growth.

As a check on the assumption of homogeneity of variance implicit in ANOVA and Williams' Test, data for each endpoint were analyzed using Bartlett's Test. The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as the Maximum Acceptable Toxicant Concentration (MATC).

12. **REPORTED RESULTS:** Water quality conditions during the 83-day study period (Table 1, attached) were satisfactory for the promotion of embryo hatchability, larval survival, and growth of rainbow trout. Table 2 (attached) presents measured concentrations of HWG 1608 Technical during the 83-day exposure period. The author stated that the concentrations measured during the initial 77 days of the study were generally consistent and varied minimally among replicate test aquaria. Only the measured concentrations during these 77-day period were used to establish the mean measured concentrations which ranged from 80 to 102% of the nominal concentrations. The author did not include the values measured at test termination (day 83) in the determination of mean measured concentrations since they were consistently low and the analyses of the quality assurance samples at this time interval resulted in recoveries of only 43 to 52%. Due to a scheduling conflict, samples collected on day 83 were stored for 48 hours prior to the analyses, while those collected on other days were analyzed within 12 hours of sample collections. Throughout the 83-day test, the diluter system functioned properly and no visible signs of undissolved material was observed in any of the exposure solutions.

A summary of the biological results for the definitive exposure of rainbow trout embryos and larvae to HWG 1608 Technical is presented in Table 5 (attached). Exposure to all concentrations of HWG 1608 Technical tested did not adversely affect the embryo viability or survival of organisms at hatch when compared to the pooled control organisms. By exposure day 42, (19 days post-hatch) nearly all exposed larvae in treatment levels of  $\leq 61$  ug/L and the controls completed their development to the swim-up stage. Development of larvae in concentrations of  $\geq 120$  ug/L appeared to be delayed by several days as compared to the



larvae in the lower treatment levels and the controls. Throughout the remainder of the exposure period, larvae at these two test concentrations generally had dark color and exhibited lethargic behavior.

At test termination, larval survival in the four highest test concentrations (i.e., 25, 61, 120, and 230 ug/L mean measured concentrations) was significantly affected as compared to the survival of the pooled control larvae. Mean survival of larvae exposed to the lowest concentration tested was unaffected as compared to the survival of the pooled control organisms. Since the larval survival was significantly reduced at the four highest concentrations, the author did not statistically compare the growth data at these levels to the growth of the control organisms. However, he concluded that the fish growth at these levels appeared to be adversely affected. Larval growth in the remaining treatment (12 ug/L) was not significantly different from that of the pooled controls. Based on these results, the no-observed-effect concentration for rainbow trout survival and growth was determined to be 12 ug/L HWG 1608.

Throughout the post-hatch exposure period of this study, larvae at the four highest test concentrations exhibited abnormal appearance (dark coloration) and behavior (lethargic, partial or complete loss of equilibrium). Organisms exposed to the lowest test concentration appeared normal in appearance and behavior when compared to larvae in the control solutions.

Based on the significantly ( $p \leq 0.05$ ) reduced larval survival after 60 days post-hatch exposure to concentrations of  $\geq 25$  ug/L HWG 1608 Technical, the maximum acceptable toxicant concentration (MATC) was estimated to be  $> 12$  ug/L and  $< 25$  ug/L (geometric mean MATC = 17 ug/L).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author. The raw data and the final report for this study were inspected by the Quality Assurance Unit of Springborn Life Sciences, Inc. to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations.



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

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

- o The total hardness of dilution water (26-36 mg/L as  $\text{CaCO}_3$ ) used in the test was slightly lower than the recommended hardness of 40-48 mg/L as  $\text{CaCO}_3$ . Also, there was no report whether the water had been analyzed for pesticides, heavy metals, and other possible contaminants.

- o The test system failed to maintain the test temperature at  $12 \pm 2^\circ\text{C}$ . During the 83-day exposure period, the temperature ranged from 13 to  $16^\circ\text{C}$ .

- o The fish were fed until the end of the test. According to the SEP, fish should not be fed for at least 24 hours prior to test termination.

- o No raw data were submitted with the report.

**B. Statistical Analysis:** The reviewer reanalyzed the embryo viability, survival of organisms at hatch, and larval survival using the analysis of variance (ANOVA) and Dunnett's Test. Since no significant differences were found between the control and carrier control for any parameter tested, the pooled data of these two controls were used to compare with the data from each test concentration. The arcsine square-root transformation was used to transform the data before performing the ANOVA. All printouts are attached. The results agreed with the author's findings.

The author excluded growth data (length and wet weight) of the four highest test concentrations from statistical analyses, and then speculated that the fish growth at those levels were affected by HWG 1608 Technical. All data should have been included in the analyses. Since no raw data were submitted with the report, the effects of HWG 1608 Technical on length and weight could not be determined.



- C. Discussion/Results: When compared to the pooled control data, no adverse effects of HWG 1608 on embryo viability and survival of organisms at hatch were found. Larval survival at test concentrations of  $\geq 25$  ug/L was significantly different from that of the pooled controls. The author must submit raw data (i.e., individual measurements) for length and weight before it can be determined if the study fulfills the guideline requirements for an early life stage fish test.

Based on larval survival, the MATC value of HWG 1608 Technical for rainbow trout was determined to be between 12 and 25 ug/L mean measured concentrations.

- D. Adequacy of the Study:

- (1) Classification: ~~Supplemental~~. *Core BL 12/20/89*
- (2) Rationale: Statistical ~~analysis~~ on length and wet weight could not ~~be~~ performed due to the lack of raw data. *Data was submitted later*
- (3) Repairability: Yes, ~~submit~~ raw data for larval length and wet weight.

15. COMPLETION OF ONE-LINER: Yes, March 31, 1989.



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Pages 9 through 11 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.
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Data listing

File: hwg

Date: 03-31-1988

FILTER: None

(After Arc Sine SQRT transformation)

Obs.	CONC	VIABILIT	EMBERSURV	LARVSURV
1	1.0000	1.0948	1.3453	1.2490
2	1.0000	1.1458	1.4289	1.1731
3	1.0000	1.1326	1.3233	1.1731
4	1.0000	1.0244	1.2490	1.3453
5	2.0000	1.2490	1.3694	1.1071
6	2.0000	1.1071	1.1071	1.1071
7	3.0000	1.2327	1.3967	0.9912
8	3.0000	1.1198	1.1873	0.8861
9	4.0000	1.0472	1.2661	0.2255
10	4.0000	1.1873	1.2661	0.3218
11	5.0000	1.1458	1.3233	0.2255
12	5.0000	1.1458	1.3030	0.3977
13	6.0000	1.1731	1.2661	0.0000
14	6.0000	1.2019	1.3233	0.2255



Date: 03-31-1988

(Embryo viability)

Factors: C	<u>Cone.</u> ( $\mu\text{g/L}$ )	N	Mean	S.D.
*		14	1.1434	0.0639
1	Pooled Controls	4	1.0994	0.0545
2	12	2	1.1781	0.1003
3	25	2	1.1762	0.0798
4	61	2	1.1172	0.0991
5	120	2	1.1458	0.0000
6	230	2	1.1875	0.0204

Dependent variable: VIABILIT

Source	df	SS (H)	MSS	F	P
Between Subjects	13	0.0531			
C (CONC)	5	0.0176	0.0035	0.790	0.5880
Subj w Groups	8	0.0356	0.0044		

### Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.099	6	1.188
2	1.178		
3	1.176		
4	1.117		
5	1.146		

Comparison	Bon- ferroni	Dunnett
1 < 2		
1 < 3		
1 < 4		
1 < 5		
1 < 6		
2 > 3		N.A.
2 > 4		N.A.
2 > 5		N.A.
2 < 6		N.A.
3 > 4		N.A.
3 > 5		N.A.
3 < 6		N.A.
4 < 5		N.A.
4 < 6		N.A.
5 < 6		N.A.

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



## Analysis of Variance

File: hwg

Date: 03-31-1988

**FILTER:** None

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

(survival at hatch)

\* Indicates statistics are collapsed over this factor

Factors: C	Conc. ( $\mu\text{g/L}$ )	N	Mean	S.D.
*		14	1.2968	0.0833
1	Pooled Controls	4	1.3366	0.0740
2	12	2	1.2383	0.1855
3	25	2	1.2920	0.1481
4	61	2	1.2661	0.0000
5	120	2	1.3131	0.0144
6	230	2	1.2947	0.0404

[illegible]

Fmax for testing homogeneity of between subjects variances: Not defined

[illegible]

Analysis of Variance                      Dependent variable: EMBRSURV

Source	df	SS (H)	MSS	F	P
Between Subjects	13	0.0903			
C (CONC)	5	0.0157	0.0031	0.336	0.8783
Subj w Groups	8	0.0746	0.0093		

[illegible]

### Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.337	6	1.295
2	1.238		
3	1.292		
4	1.266		
5	1.313		

Comparison	Bon- ferroni	Dunnett
1 > 2		
1 > 3		
1 > 4		
1 > 5		
1 > 6		
2 < 3		N.A.
2 < 4		N.A.
2 < 5		N.A.
2 < 6		N.A.
3 > 4		N.A.
3 < 5		N.A.
3 < 6		N.A.
4 < 5		N.A.
4 < 6		N.A.
5 > 6		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).



Date: 03-31-1988

(Charwal survival)

Factors:	C	<u>Conc.</u> (mg/L)	N	Mean	S.D.
*			14	0.7449	0.4794
1		Pooled Controls	4	1.2351	0.0817
2		12	2	1.1071	0.0000
3		25	2	0.9387	0.0743
4		61	2	0.2736	0.0681
5		120	2	0.3116	0.1218
6		230	2	0.1128	0.1595

Dependent variable: LARVSURV

Source	df	SS (H)	MSS	F	P
Between Subjects	13	2.9881			
C (CONC)	5	2.9176	0.5835	66.275	0.0000
Subj w Groups	8	0.0704	0.0088		

### Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.235	6	0.113
2	1.107		
3	0.939		
4	0.274		
5	0.312		

Comparison	Bon- ferroni	Dunnett
1 > 2		
1 > 3	0.0991	0.0500
1 > 4	0.0000	0.0100
1 > 5	0.0000	0.0100
1 > 6	0.0000	0.0100
2 > 3		N.A.
2 > 4	0.0004	N.A.
2 > 5	0.0006	N.A.
2 > 6	0.0000	N.A.
3 > 4	0.0018	N.A.
3 > 5	0.0027	N.A.
3 > 6	0.0004	N.A.
4 < 5		N.A.
4 > 6		N.A.
5 > 6		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).



Shawnee No. 128997

Study/Species/Lab/  
Succession

Chemical  
Active

Chemical Name Ethyltriethyl  
(HWG 1608 Technical)

Results

Page 1 of 1

Reviewer/  
Date

Validat  
Status

Avian Reproduction,

Species:

Lab:

Acc\*;

Group Dose (ccm) Effect/Parameters Mort. (%) %Ch. Inh.

Control

Treatment I

Treatment II

Treatment III

Study Duration:

Comments:

Field Study (Simulated/Actual)

Species:

Lab:

Acc.\*;

Group Rate (ai/a) Treatment Interval Total # Treatments Mort. (%)

Control

Treatment I

Treatment II

Treatment III

Crop/Site:

Study Duration:

Comments:

Chronic fish,

Species Salmo gairdneri  
96.3

Lab: Springborn Life Sciences

Acc.\*; 407009-14

Concentrations Tested (ppb) = 12, 25, 61, 120, 230

MATC = > 12 < 25 ppb\*

Effect Parameter = Larval Survival

Conc. Mort. (%) = 10

Sol. Conc. Mort. (%) = 13

AK/3-31-89 Suppl.

Comments: \* Mean measured concentrations

Chronic invertebrate

Species

Lab

Acc.\*

Concentrations Tested (pp) =

MATC => < pp

Effect Parameter(s)

Conc. Mort. (%) =

Sol. Conc. Mort. (%) =

Comments: