Accession No. 408937-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Sulfosate

2. **TEST MATERIAL:** SC-0224 Technical; Lot No. WRC-10387-47-1; Sample purity 57.3%

3. **STUDY TYPE:** Early Life Stage Toxicity Test
   Species Tested: Salmo gairdneri

4. **CITATION:** Cohle, Paul. (1988); Early Life Stage Toxicity of SC-0224 Technical to Rainbow Trout (Salmo gairdneri) in a Flow-Through System, Report No. 35819. Prepared by Analytical Bio-Chemistry Laboratories, Inc. Columbia, Missouri; submitted by ICI Agrochemicals, Surrey, UK; Accession No. 408937-04.

5. **REVIEWED BY:**

   Kimberly D. Rhodes
   Aquatic Toxicologist
   Hunter/ESE

   Signature: Kimberly D. Rhodes
   Date: 1/9/89

6. **APPROVED BY:**

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

   Signature: P. Kosalwat
   Date: January 12, 1989

   Henry T. Craven
   Supervisor, EEB/HED
   USEPA

   Signature: Henry T. Craven
   Date: 1/30/89

7. **CONCLUSIONS:** This study appears to be scientifically sound, but does not fulfill the guideline requirements for a fish early life stage toxicity test. Based on a significant reduction in growth after 60 days of exposure, the MATC of SC-0224 for rainbow trout (Salmo gairdneri) was between 51 and 100 mg a.i./L.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Unfertilized rainbow trout eggs (Salmo gairdneri) and semen were obtained from a commercial supplier in California. Upon receipt, the eggs were at a temperature of approximately 11.5°C. The eggs were poured into a dry plastic bowl which was resting in an 11°C water bath. The sperm was poured over the eggs and they were mixed gently by hand. An egg wash solution (slightly saline) was added to the bowl and the eggs were again stirred. The eggs were allowed to stand in this solution for approximately 30 seconds. Excess liquid and sperm were poured off followed by a fresh ABC soft reconstituted water rinse. At 15 minute intervals for the next hour and a half, aliquots of test dilution water (ABC well water) were added to the bowl containing the fertilized eggs in soft water. The eggs were then ready to be added to the test chambers.

B. **Test System:** A proportional diluter system described by Mount and Brungs, utilizing a Hamilton Micro Lab 420 syringe dispenser, was used for the intermittent introduction of SC-0224 Technical test solutions and diluent water into each test chamber. The proportional diluter system used for the project was set to provide test levels approximately 50 percent dilutions of each other. The diluter delivered an average rate of approximately 57 mL/minute/replicate of test solution or control water to the test vessels which was sufficient to replace a replicate volume 7.1 times in a 24 hour period over the course of the study. Five concentrations of the test material with a dilution water control were tested. The test chambers were immersed in a temperature controlled water bath held at 10 $\pm$ 1°C. The lighting was maintained on a 16-hour daylight photoperiod, after the embryos had hatched.

The rainbow trout eggs were incubated in cups suspended in the treatment and control water. These egg incubation cups were made from 8-cm diameter glass jars with the bottoms cut out and stainless steel screening (16 mesh) fused to the bottom. To insure exchange of water, the egg cups were oscillated in the test solution and/or control water by means of a rocker arm apparatus driven by a 2 rpm electric motor.

Dilution water for the rainbow trout test was well water characterized as having a pH of 7.9 - 8.4, total hardness of 250 - 284 mg/L as $CaCO_3$, total alkalinity of 330 - 368 mg/L $CaCO_3$ and specific conductance of 554 - 645 umhos/cm.

C. **Dosage:** 60-day flow-through post-hatch early life stage test.

D. **Design:** Thirty rainbow trout eggs were randomly introduced into each quadruplicate chamber (120 eggs per concentration). When hatching commenced, the number of eggs hatched in each incubation cup was recorded daily until hatching was completed. The 60-day post hatch growth period began when hatch was greater than 95 percent. At 11 days post-hatch the rainbow trout sac fry were transferred from the egg incubation cups into growth chambers. Fry growth data were collected on days 35 and 60 post-hatch. These same days were also used as data points for survival analysis, since the most accurate counts of the fish could be made on these days. Feeding began after 13 days post-hatch. The fry were fed brine shrimp nauplii (Artemia salina) throughout the study. Salmon starter in pellet form was added to the diet after 25 days post-hatch. Water quality parameters of pH and conductivity were measured on Day 0, Day 1, Day 7 and on every 7th day thereafter until test termination from the control, low concentration, and high concentration. Dissolved oxygen was measured on Day 0, Day 1, Day 7, and on every 7th day thereafter until test termination in all test concentrations. Water hardness and alkalinity were measured on Day 0, Day 48, Day 76, and Day 91. Temperature was monitored daily and was also continuously recorded with a temperature data logger. A control and five nominal SC-0224 concentrations of 6.0, 12, 25, 50, and 100 mg/L based on active ingredient were tested. The measured concentrations of SC-0224 in test water were determined on days 0, 1, 14 and every 7 days thereafter during the study until test termination.

E. **Statistics:** Comparison analyses between the control and five test levels were carried out using the measured parameters of hatchability, survival, standard length and wet weight by analysis of variance (ANOVA).

Prior to evaluation of growth data by ANOVA, consideration was given to the need for any data transformations. Homogeneity of variances among groups were evaluated using Bartlett's test. Bartlett's tests showed that error variances were within the statistical criterion; therefore, no data transformations were required.

One-way analysis of variance (ANOVA) calculations were used to determine if significant differences existed. The data were analyzed by comparing all replicates (24 total) of the control and 5 test levels against each other and by combining the data from the 4 replicates within a concentration into a single group and comparing the concentrations against each other. If treatment effects were indicated by a significant F-test of the mean square ratios, Tukey's HSD multiple means comparison test was used to determine which exposure levels differed from the control values.

Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. Differences were determined by ANOVA.

12. **REPORTED RESULTS**: Hatchability of eyed rainbow trout eggs after 35 days of continuous exposure to SC-0224 technical ranged from 74 to 89% in the control and 5 test levels. No statistical significant reduction in hatch was found between the control and treatment levels. The survival of trout fry continuously exposed to SC-0224 technical after 70 and 95 days (35 and 60 days post-hatch) is shown in Table 10 (attached). No significant reduction in fry survival was detected at either the 35 or 60 day post-hatch survival analysis points.

A statistically significant reduction in growth was detected in the lowest mean measured concentration (6.8 mg/L) at both the 35 (length) and 60 day (length and weight) post-hatch analysis points (Table 11, attached). It appeared to be traceable primarily to the C replicate of this concentration. No reduction was detected in fish from the other concentrations, therefore, it did not appear to be part of a toxicant dose-response pattern and was considered as aberrant. Day 60 post-hatch analysis also revealed a statistically significant growth reduction in the highest nominal concentration (100 mg/L). Low dissolved oxygen concentrations in the highest concentration near the end of the study may have influenced both this reduction as well as certain acute effects observed during the last 8 days of the study.

Based on the reduction in growth after 60 days of exposure in 100 mg/L, the Maximum Acceptable Toxicant Concentration (MATC) range is estimated to be between 51 and 100 mg/L. The no-observable effect concentration appeared to be at the mean measured SC-0224 technical concentration of 51 mg/L based on active ingredient.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The Maximum Acceptable Toxicant Concentration (MATC) range is estimated to be between 51 and 100 mg/L. The no-observable effect level appeared to be at the mean measured SC-0224 technical concentration of 51 mg/L based on active ingredient.

The data were audited by the laboratory's Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and pertinent EPA Good Laboratory Practice (GLP) Regulations. A GLP compliance statement was included and signed by the Quality Assurance Unit.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

- The SEP specifies that the report of the results must include raw data on hatchablility, survival, standard length, and wet weight of the rainbow trout eggs and fry in order to confirm statistical analysis. The reviewer could not confirm statistical analysis due to lack of raw data.

- The SEP recommends that test water should have a hardness of 40 to 48 mg/L as $CaCO_3$ and a pH range of 7.2 to 7.6. The hardness of the test water in this study was 250-284 mg/L $CaCO_3$ and the pH ranged from 7.9 to 8.4.

- The SEP states that the flow rate must be capable of maintaining the dissolved oxygen concentration at above 75 percent of saturation. By day 84 the dissolved oxygen concentration in the three highest test levels were 7.5, 6.9, and 5.5 mg/L which represented 69, 64 and 51% oxygen saturation at $10^{o}C$.

- It is not clear from the report how the water samples were collected. Were samples collected from splitter boxes or composited from the replicate aquaria?

B. **Statistical Analysis:** The reviewer could not confirm statistical analysis due to the lack of raw data.

C. **Discussion/Results:** The study results appear scientifically valid. However, lack of raw data on the hatchability, survival, standard length, and wet weight of the rainbow trout eggs or fry prevent the validation of this study. In addition, the sample collection procedure should be clarified.

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D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** See comments in section 14.

(3) **Repairability:** Yes. Submission of appropriate raw data.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 01-09-89.

Study No. _____ Chemical Name Sulfosate Chemical Class _____ Page 2 of _____

SC-0224 Technical

Study/Species/Lab/ Succession	Chemical % Active	Results				Reviewer/ Date	Validation Status
Avian Reproduction,		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Chg Inh.	
Species:		Control					
		Treatment I					
Lab:		Treatment II					
		Treatment III					
Acc*;		Study Duration:					
		Comments:					

Field Study(Simulated/Actual)	Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)		
Species:	Control						
	Treatment I						
Lab:	Treatment II						
Acc.*;	Treatment III						
	Crop/Site:		Study Duration:				
	Comments:						

Chronic fish,

Species Salmo gairdneri

Lab: Analytical Bio-Chemistry 57.3%

Acc.*; 408937-04

Concentrations Tested (ppm)= Control 6.8, 12, 23, 51, 101

MATC = >51 <100 ppm. Effected Parameter = growth

Contr. Mort.(%)= 8% Sol. Contr. Mort.(%)= N/A K.R. Suppl 1/9/89

Comments: Based on mean measured concentrations

Chronic invertebrate

Species _____

Lab _____

Acc.* _____

Concentrations Tested (pp__)= _____

MATC =>____< ____pp__. Effected Parameter(s) _____

Contr. Mort.(%)=_____ Sol. Contr. Mort.(%)=_____

Comments:

Sulfosate ecological effects review

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___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

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