Accession No. 408937-04 and
411114-02 (Supplement)

DATA EVALUATION RECORD

1. CHEMICAL: Sulfosate.
Shaughnessey No. 128501.

2. TEST MATERIAL: SC-0224 Technical; Lot No. WRC-10387-47-01; Sample purity 57.3%; a light amber colored liquid.

3. STUDY TYPE: Early Life Stage Toxicity Test.
Species Tested: Rainbow trout (Salmo gairdneri).

4. CITATION: Cohle, P. 1988. Early Life Stage Toxicity of SC-0224 Technical to Rainbow Trout (Salmo gairdneri) in a Flow-Through System. Prepared by Analytical Bio-Chemistry Laboratories, Inc. Columbia, Missouri. Report No. 35819. Submitted by ICI Americas Inc., Wilmington, Delaware. Accession No. 408937-04 and 411114-02 (Supplement).

5. REVIEWED BY:

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Kimberly D. Rhodes
Date: July 10, 1989

6. APPROVED BY:

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

Signature: P. Kosalwat
Date: July 11, 1989

Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven 9/11/89
Date: Daniel Rathman

7. CONCLUSIONS: This study appears to be scientifically sound and fulfills the guideline requirements for a fish early life stage toxicity test. Based on a significant reduction in growth (length and weight), the MATC of SC-0224 for rainbow trout (Salmo gairdneri) was > 51 < 101 mg a.i./L mean measured concentration.

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. Each glass test aquarium measured 30.7 x 15.8 cm with a water depth of 24 cm, yielding an approximate 11.6 liters. 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 ± 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: 95-day flow-through 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 on day 13 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 a.i./L were tested. The measured concentrations of SC-0224 in test water were determined on days 0, 1, 7, 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 was 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 (arcsine square-root percentage) transformation of the data. Differences were determined by ANOVA.

12. REPORTED RESULTS: The mean measured concentrations of SC-0224 Technical maintained during this chronic study were 6.8, 12, 23, 51, and 100 mg/L, which ranged from 88% to 110% of the nominal concentrations. The water quality parameters remained within acceptable ranges. Mean dissolved oxygen saturation was $\geq$ 75% in all test levels through day 70. By day 84 the mean dissolved oxygen concentrations of the three highest concentrations slightly fell to 69%, 64% and 51% oxygen saturation, respectively. The pH values of the test solutions ranged from 7.9 to 8.4 and were generally consistent between control, low and high test levels throughout the duration of the study. Hardness ranged from 250 to 284 mg/L and alkalinity from 330 to 368 mg/L. The temperature range from the thermometer recordings was 9 to 11.1°C.

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. Swim-up began on day 48 (13 days post-hatch) and was for the most part complete by day 57. There did not appear to be a dose-related effect when time to swim up in the control fry was compared to that in fry

from the treated 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 a.i./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 a.i./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. During the final 8 days of the study, some of the 101-mg a.i./L fish began to show an increase in abnormal observations, including fish on the bottom of the test chambers, flaring of the opercula on one side, exophthalmia, dark discoloration, surfacing, loss of equilibrium and bloated abdomen.

Based on the reduction in growth after 60 days of exposure in 101 mg a.i./L mean measured concentration, the Maximum Acceptable Toxicant Concentration (MATC) range is estimated to be between 51 and 101 mg a.i./L mean measured concentration. The no-observable effect concentration appeared to be at the mean measured SC-0224 technical concentration of 51 mg a.i./L.

13. <u>STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES</u>:
Based on the reduction in growth after 60 days of exposure in 101 mg a.i./L mean measured concentration, the Maximum Acceptable Toxicant Concentration (MATC) range is estimated to be between 51 and 101 mg a.i./L mean measured concentration. The no-observable effect concentration appeared to be at the mean measured SC-0224 technical concentration of 51 mg a.i./L.

A GLP compliance statement was included in the report and the study was audited by a QA unit. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good

Laboratory Practice Standards: Pesticide Programs (40 CFR 160).

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:

o 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.

o 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 a.i./L which represented 69, 64 and 51% oxygen saturation at $10^{o}C$.

o There appears to be a discrepancy in the report concerning the mean measured concentration of the highest treatment level. Table 4 states that the mean measured concentration for the highest treatment level is 100 mg a.i./L while Tables 10 and 11 state that it is 101 mg a.i./L. The reviewer believes that 101 mg a.i./L is the correct value.

B. Statistical Analysis: The reviewer evaluated embryo hatchability and larval survival following an arc-sine square-root transformation of the data. The growth data (standard length and wet weight) were statistically evaluated by ANOVA without any transformations. All printouts are attached.

The reviewer confirmed no significant difference between survival of larvae and hatchability of embryos in any treatment level and the control.

The reviewer confirmed a significant difference at P = 0.01 of length and weight of rainbow trout larvae in the lowest (6.8 mg a.i./L) and highest (101 mg a.i./L) mean measured concentration when compared to the control. The significant difference at the lowest concentration is not believed to be a toxicant related effect since there was no effect in the next three higher concentrations.

C. Discussion/Results: This study appears scientifically valid. Based on a significant reduction in growth (length and weight), the MATC of SC-0224 Technical for rainbow trout (Salmo gairdneri) was > 51 < 101 mg a.i./L mean measured concentration.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 6-30-89.

Sulfosate ecological effects review

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Pages 8 through 11 are not included in this copy.

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SC-0224 Technical
Length

Analysis of Variance File: SCGROW Date: 06-30-1989

FILTER: None

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

* Indicates statistics are collapsed over this factor

Factors: C	R	Concentration mg/L	N	Mean	S.D.
*	*		316	3.6541	0.4076
1	*	Control	55	3.8200	0.2870
2	*	6.8	52	3.5404	0.4225
3	*	12	52	3.6750	0.3688
4	*	23	58	3.8414	0.2859
5	*	51	52	3.7538	0.4056
✱ 6	*	101	47	3.2213	0.3375
*	1		79	3.6430	0.3640
*	2		80	3.6425	0.4142
*	3		76	3.5895	0.4568
*	4		81	3.7370	0.3858
1	1		14	3.9000	0.3637
1	2		14	3.8286	0.3099
1	3		14	3.8214	0.2359
1	4		13	3.7231	0.2127
2	1		15	3.5133	0.3335
2	2		12	3.6083	0.3777
2	3		11	3.2000	0.4382
2	4		14	3.7786	0.3847
3	1		14	3.7571	0.1604
3	2		15	3.7867	0.3543
3	3		13	3.4692	0.4029
3	4		10	3.6600	0.4789
4	1		15	3.8600	0.1682
4	2		14	3.8500	0.3459
4	3		14	3.7643	0.3079
4	4		15	3.8867	0.3114
5	1		9	3.4556	0.2603
5	2		13	3.4538	0.4235
5	3		15	3.9667	0.2554
5	4		15	3.9800	0.3121
6	1		12	3.2417	0.3655
6	2		12	3.2417	0.3655
6	3		9	2.9778	0.2167
6	4		14	3.3429	0.3005

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Fmax for testing homogeneity of between subjects variances: 8.92

Number of variances= 24 df per variance= 12.

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Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	315	52.3447			
C (CONC)	5	13.5653	2.7131	25.303	0.0000
R (REP)	3	1.3028	0.4343	4.050	0.0076
CR	15	6.1674	0.4112	3.835	0.0000
Subj w Groups	292	31.3091	0.1072		

SC-0224 Technical
(Length)

Analysis of Variance File: SCGROW Date: 06-30-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	3.820	6	3.221
2	3.540		
3	3.675		
4	3.841		
5	3.754		

Comparison	Dunnett
1 > 2	0.0100
1 > 3	
1 < 4	
1 > 5	
* 1 > 6	0.0100
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).

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Post-hoc tests for factor R (REP)

Level	Mean
1	3.643
2	3.642
3	3.589
4	3.737

Comparison	Dunnett
1 > 2	
1 > 3	
1 < 4	
2 > 3	N.A.
2 < 4	N.A.
3 < 4	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).

SC-0224 Technical
Weight

Analysis of Variance File: SCGROW Date: 06-30-1989

FILTER: None

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

* Indicates statistics are collapsed over this factor

Factors: C	R	Concentration mg/L	N	Mean	S.D.
*	*		316	0.8071	0.2417
1	*	Control	55	0.8951	0.2007
2	*	6.8	52	0.7618	0.2507
3	*	12	52	0.8072	0.2158
4	*	23	58	0.9074	0.1868
5	*	51	52	0.8604	0.2397
✱ 6	*	101	47	0.5712	0.2007
*	1		79	0.8015	0.2089
*	2		80	0.8093	0.2411
*	3		76	0.7688	0.2684
*	4		81	0.8461	0.2442
1	1		14	0.9551	0.2209
1	2		14	0.9168	0.2284
1	3		14	0.8669	0.1942
1	4		13	0.8373	0.1473
2	1		15	0.7324	0.1922
2	2		12	0.8014	0.2277
2	3		11	0.5585	0.2197
2	4		14	0.9190	0.2485
3	1		14	0.8266	0.1137
3	2		15	0.8618	0.2085
3	3		13	0.7111	0.2464
3	4		10	0.8232	0.2784
4	1		15	0.8967	0.1407
4	2		14	0.9171	0.2169
4	3		14	0.8769	0.1925
4	4		15	0.9374	0.2053
5	1		9	0.7072	0.1650
5	2		13	0.6886	0.2180
5	3		15	0.9925	0.1802
5	4		15	0.9691	0.2162
6	1		12	0.6312	0.2366
6	2		12	0.6312	0.2366
6	3		9	0.4156	0.0900
6	4		14	0.5682	0.1394

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Fmax for testing homogeneity of between subjects variances: 9.58

Number of variances= 24 df per variance= 12.

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Analysis of Variance Dependent variable: WEIGHT

Source	df	SS (H)	MSS	F	P
Between Subjects	315	18.4092			
C (CONC)	5	3.8794	0.7759	18.809	0.0000
R (REP)	3	0.3498	0.1166	2.827	0.0385
CR	15	2.1345	0.1423	3.450	0.0000
Subj w Groups	292	12.0454	0.0413		

SC-0224 Technical
Weight

Analysis of Variance File: SCGROW Date: 06-30-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	0.895	6	0.571
2	0.762		
3	0.807		
4	0.907		
5	0.860		

	Comparison	Dunnett
	1 > 2	0.0100
	1 > 3	
	1 < 4	
	1 > 5	
*	1 > 6	0.0100
	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).

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Post-hoc tests for factor R (REP)

Level	Mean
1	0.802
2	0.809
3	0.769
4	0.846

Comparison	Dunnett
1 < 2	
1 > 3	
1 < 4	
2 > 3	N.A.
2 < 4	N.A.
3 < 4	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).

SC-0224 Technical
Survival

Analysis of Variance File: scsurv Date: 06-30-1989

FILTER: None

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

* Indicates statistics are collapsed over this factor

Factors: C Concentration mg/L	N	Mean	S.D.
*	24	1.2823	0.2086
1 control	4	1.2777	0.0505
2 6.8	4	1.2513	0.2429
3 12	4	1.2957	0.2001
4 23	4	1.4369	0.1546
5 51	4	1.3074	0.3303
6 101	4	1.1245	0.1779

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Fmax for testing homogeneity of between subjects variances: 42.71
Number of variances= 6 df per variance= 3.

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Analysis of Variance Dependent variable: SURV

Source	df	SS (H)	MSS	F	P
Between Subjects	23	1.0012			
C (CONC)	5	0.2023	0.0405	0.912	0.4990
Subj w Groups	18	0.7989	0.0444		

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Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.278	6	1.125
2	1.251		
3	1.296		
4	1.437		
5	1.307		

Comparison	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).

SC-0224 Technical
hatchability

Analysis of Variance   File: scsurv   Date: 06-30-1989

FILTER: None

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

* Indicates statistics are collapsed over this factor

Factors:	C Concentration (mg/L)	N	Mean	S.D.
	*	24	1.1673	0.1475
	1 Control	4	1.2199	0.1073
	2 6.8	4	1.1746	0.1101
	3 12	4	1.1213	0.0891
	4 23	4	1.2844	0.2375
	5 51	4	1.1746	0.1333
	6 101	4	1.0289	0.1025

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Fmax for testing homogeneity of between subjects variances: 7.10
Number of variances= 6 df per variance= 3.

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Analysis of Variance   Dependent variable: HATCH

Source	df	SS (H)	MSS	F	P
Between Subjects	23	0.5003			
C (CONC)	5	0.1515	0.0303	1.563	0.2190
Subj w Groups	18	0.3488	0.0194		

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Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.220	6	1.029
2	1.175		
3	1.121		
4	1.284		
5	1.175		

Comparison	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).

Shaughnessey No. 128501 Chemical Name Sulfosate (SC-0224 Technical) Chemical Class _____ Page _____ of _____

Study/Species/Lab/ Succession	Chemical % Active	Results				Reviewer/ Date	Valid: Stat
Avian Reproduction,		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	1C% 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 Laboratories, Inc. 57.3% Acc. 408937-04

Mean Concentrations Tested (ppm)= 6.8; 12; 23; 51; 101

MATC = >51 <101 ppm. Effected Parameter = length and weight

Contr. Mort.(%)= 8% Sol. Contr. Mort.(%)= N/A

A.R. 6/30/89 Core

Comments: Based on mean measured concentrations. Corrected for active ingredient.

Chronic invertebrate Species _____ Lab _____ Acc. _____

Concentrations Tested (pp__)= _____

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

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

Comments: