Accession No. 408937-05

DATA EVALUATION RECORD

1. **CHEMICAL:** Sulfosate Technical

2. **TEST MATERIAL:** SC-0224 Technical; Lot No. WRC 9031-49-1
   Sample purity 56.2%

3. **STUDY TYPE:** Life Cycle Test with Freshwater Invertebrate
   Species Tested: Daphnia magna

4. **CITATION:** Forbis, A.D. (1987) Chronic Toxicity of SC-0224 to Daphnia magna Under Flow-Through Test Conditions, ABC Study No. 35820. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri; submitted by ICI Americas Inc., Farmington, Connecticut; Accession No. 408937-05.

5. **REVIEWED BY:**

   Kimberly D. Rhodes
   Aquatic Toxicologist
   ESE

   Signature: Kimberly D. Rhodes
   Date: 01/09/89

6. **APPROVED BY:**

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

   Signature: P. Kosalwat
   Date: January 11, 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 freshwater invertebrate life cycle test. The MATC of SC-0224 for Dalphnia magna was between 1.2 and 2.1 mg/L based on statistical reductions in length of adult D. magna and in mean young produced per adult. A 21-day EC50 was calculated to be 2.6 mg/L.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS**:

A. **Test Animals**: The daphnids (*Daphnia magna*) used to initiate the life cycle test exposure were obtained from the culture unit at the testing facility. All daphnids were cultured and tested in a temperature controlled area at 20 ± 2°C. The lighting was 50-70 footcandles on a 16-hour daylight photoperiod, 8-hour darkness and 30-minute transition periods. During the holding period, the daphnids were fed a suspension of algae (*Selenastrum capricornutum*) supplemented with a Tetramin/cereal leaves/yeast suspension. Only first-instar daphnids (<24 hours old) were selected for testing.

B. **Test System**: The test was conducted in a half-liter proportional diluter system described by Mount and Brungs (1967), utilizing a Hamilton Micro Lab 420 syringe dispenser. A dilution factor of 50 percent was used. The diluter delivered five concentrations of SC-0224 and a dilution water control to four replicate one-liter test aquaria. The diluter provided for approximately 6.1 volume replacements per 24-hour period. Illumination was provided by fluorescent lights set on a 16-hour light and 8-hour dark and 30-minute transition photoperiod. Test temperature was maintained at 20 ± 2°C by a temperature controlled water bath.

The water parameters of the well water used as dilution water were, total hardness: 206-275 mg/L as $CaCO_3$; total alkalinity: 224-336 mg/L as $CaCO_3$; pH: 7.6 to 8.4; dissolved oxygen: 7.4 - 9.1 mg/L; and conductivity: 500-650 umhos/cm.

C. **Dosage**: Twenty-one day flow-through life cycle test.

D. **Design**: Forty *D. magna* (≤ 24 hours old) were impartially distributed to each test concentration (10 per quadruplicate) to initiate the test. A control and nominal SC-0224 concentrations of 0.27, 0.54, 1.1, 2.2, and 4.5 mg/L were used. Exposure concentrations of SC-0224 were radiochemically measured on days 0, 4, 7, 14, and 21. The mean measured test concentrations reported were 0.34, 0.65, 1.2, 2.1 and 4.8 mg/L. Water quality parameters of dissolved oxygen and pH were measured on days 0, 4, 7, 14 and 21 in two alternating replicates of the control, low, middle and high test concentrations. Temperature measurements of the water bath were made daily and were recorded continuously with a data logger.

E. Statistics: The selected parameters of survival, adult length (pooled) and total young/adult/reproduction day were analyzed using a one-way analysis of variance. When treatment effects were indicated following a significant F-test of the mean square ratios a multiple means comparison test, Dunnett's Test, was used to determine which exposure levels differed from the control value. Percent survival data were transformed for analysis. All differences were considered significant at the 95% confidence level. Total young/adult/reproduction day for each replicate was calculated by dividing the total number of young produced by the total number of adult reproduction days. The number of reproduction days (normally 13-15) were counted from the day instars were first observed, which for this study ranged from Day 9 to Day 12. Adult reproduction days were calculated for each change in survival in order to be corrected for mortality. Total number of adult reproduction days for each replicate was the sum of each adult reproduction day for each change in survival. The 21-day EC50 was calculated by employing a computerized EC50 program developed by Stephan et al.

12. REPORTED RESULTS: A summary of the biological results of the exposure of daphnids to SC-0224 is provided in the Table 3 (attached). The mean measured concentrations of SC-0224 during the 21-day exposure ranged from 95 to 126% of expected nominal concentrations. Statistical analysis of survival for Daphnia magna after a 21-day exposure to SC-0224 indicated that daphnid survival was significantly different from the control in the SC-0224 mean measured concentration of 4.8 mg/L. The MATC limits for survival were estimated to be 2.1 and 4.8 mg/L. A 21-day EC_{50} was calculated to be 2.7 mg/L.

The daphnids lengths in SC-0224 mean measured concentrations of 2.1 and 4.8 mg/L were significantly different ($P \leq 0.05$) from the control. The MATC limits for length were estimated to be between 1.2 and 2.1 mg/L. Length measurements could not be made on the 4.8 mg/L test level daphnids since all had died by day four.

The mean young/adult/reproduction day for 21 days were significantly affected in mean measured concentrations of 2.1 and 4.8 mg/L of SC-0224. The estimated MATC limits for reproduction were 1.2 and 2.1 mg/L. Mean young/adult/reproduction day for 21 days could not be calculated for the 4.8 mg/L test level since all daphnids had died before reaching a reproductive state.
Based on the statistical analysis of survival, mean adult length and young/adult/reproduction day, from this 21-day

Daphnia magna dynamic life cycle study, the MATC limits were estimated to be between 1.2 and 2.1 mg/L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: Based on the statistical analysis of survival, mean adult length and young/adult/reproduction day, from this 21-day Daphnia magna dynamic life cycle study, the MATC limits were estimated to be between 1.2 and 2.1 mg/L.

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 overall procedures appear to be in accordance with the Guidelines. However, the SEP specifies that "A report of the results of a test must include data on the survival of first generation daphnids, production of young by first generation daphnids at various times for each treatment, and the length of first generation daphnids at the end of test." The above raw data were not presented by the author. In addition, it is not clear whether the duplicate water samples collected from each treatment were collected from the splitter boxes or from individual test aquaria.

B. Statistical Analysis: The reviewer used the Toxanal computer program to calculate the LC50 values. These calculations are attached. The probit method provides a 48-hour LC50 value for SC-0224 of 2.6 mg/L with a 95 percent confidence interval of 0 and infinity which is similar as that reported by the author. Validation of the survival, reproduction, and growth data could not be conducted because of the lack of raw data.

C. Discussion/Results: The study results appear scientifically valid. However, lack of data on the survival, reproduction, and growth of the first generation daphnids at test termination affect the validation of this study. In addition, the sample collection procedure should be clarified. The 21-day EC_{50} was calculated to be 2.6 mg/L.

D. **Adequacy of the Study**:

(1) **Classification:** Supplemental

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

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

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

Study/Species/Lab/ Succession	Chemical % Active	Results					Reviewer/ Date	Validation Status
Avian Reproduction,		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Che 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						
		Treatment III						
Acc.*;		Crop/Site:		Study Duration:				
		Comments:						
Chronic fish,		Concentrations Tested (pp__)= _____						
Species		MATC = >___ <___ pp___.		Effected Parameter = _____				
Lab:		Contr. Mort.(%)= _____		Sol. Contr. Mort.(%)= _____				
Acc.*;		Comments:						
Chronic invertebrate		Concentrations Tested (ppm)= Control 0.34, 0.65, 1.2, 2.1, 4.8 ppm						
Species Daphnia magna		MATC =>1.2 < 2.1 pp m.		Effected Parameter(s) length in adult D. Magna				
Lab Analytical Bio-Chemistry	56.2%	Contr. Mort.(%)= 8%		Sol. Contr. Mort.(%)= N/A mean young produced			K.R.	Suppl
Acc.* 408937-05		Comments: 21-Day EC50 = 2.6 ppm		per adult			1/9/89	
		Based on mean measured concentrations						

TABLE 3

Percent Survival, Adult Length and Young/Adult/Reproduction Day of Daphnia magna Continuously Exposed to ^{14}C-SC-0224 During a 21-Day Life Cycle Study

Chamber I.D. (nominal concentrations)	Mean Measured Concentration (mg/l)	Mean[a] Percent Survival	Adult[a] Mean Length (mm)	Mean[a] Young/Adult/ Reproduction Day
Control	---	92	4.0	5.5
Level #1 (0.27 mg/l)	0.34	98	3.9	5.4
Level #2 (0.54 mg/l)	0.65	100	4.0	5.4
Level #3 (1.1 mg/l)	1.2	100	3.9	5.2
Level #4 (2.2 mg/l)	2.1	80	3.8[b]	3.4[b]
Level #5 (4.5 mg/l)	4.8	0[b]	0[b]	0[b]

[a]All mean values are calculated directly from the raw data.

[b]Denotes values significantly different ($\alpha \leq 0.05$) from the pooled controls using one-way analysis of variance (ANOVA) and Dunnett's Multiple means test.