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7200 E. ABC Lane, Columbia, MO 65202 • Tel: 573/474-8579 Fax: 573/443-9033

STUDY TITLE

7-Day Survival and Growth Tests of Dust Suppression Products EK-35 and EnviroKleen to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static Renewal Conditions

SPONSOR

Midwest Industrial Supply
1101 3rd Street Southeast
Canton, Ohio 44707

AUTHOR

Chris Hughes
Associate Scientist

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PERFORMING LABORATORY

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

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SIGNATURE PAGE

Submitted by: ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Prepared by:

Chris Hughes 03 Sep, 03

Chris Hughes
Associate Scientist
ABC Laboratories, Inc.

Date

ACUTE TOXICITY COMPENDIUM

Subject: 7-Day Survival and Growth Tests of Dust Suppression Products EK-35 and EnviroKleen to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static Renewal Conditions

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Test Substances: EK-35

Definitive Test Concentrations: EK-35: 0 (control), 5.0, 10, 20, 40, and 80 mg/L

Solution Preparation: WAF (Water Accommodated Fraction), Stirred overnight

Definitive Test Dates: August 6 to 13, 2003

Duration of Test: 7 days

Organism Source: In-house cultures

Age at Initiation: 57 days post-hatch

Test Procedures and Conditions:

Temperature:	12 ± 2°C
Lighting:	Ambient laboratory lighting, 16:8-hr light:dark
Observations:	Days 1, 2, 3, 4, 5, 6, and 7
Test chambers:	4-L glass containers
Volume per chamber:	3.0 L
Replicates per treatment:	4
Organisms per chamber:	5
Organisms per treatment:	20
Dilution water:	Blended freshwater
Solution renewal:	Daily
Aeration:	60-100 bubbles/minute

Methods:

U.S. EPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed. U.S. Environmental Protection Agency, EPA/84/R-02/012.

U.S. EPA. 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms, Fourth Edition. EPA/821/R-02/013. 335 p.

U.S. EPA. 1994. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Marine and Estuarine Organisms, Third Edition. EPA/821/R-02/014.

Toxcalc™ Version 5.0, Release 6.12. Copyright 1994 by Tidepool Scientific Software: Michael A. Ives.

Results:

Rainbow Trout 7-Day Static Renewal Test with EK-35 Cumulative Percent Mortality							
Nominal Loading Concentration (mg/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	0	0	0	0	0	0	0
5.0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
20	0	0	0	10	10	10	35
40	65	75	80	90	95	95	95
80	100	100	100	100	100	100	100

Note: Five fish per replicate, 20 fish per treatment.

Rainbow Trout 7-Day Static Renewal Test with EK-35 Mortality and Growth		
Nominal Loading Concentration (mg/L)	Mortality at 7 Days (%)	Mean Weight per Fish (mg)
Control	0	153.5
5.0	0	174.7
10	0	151.5
20	35	110.2
40	95	115.2
80	100	NA

Rainbow Trout 7-Day Static Renewal Test with EK-35 Water Quality Ranges			
Solution ID	Temperature (°C)	Dissolved Oxygen as mg/L (% Saturation)	pH
New Solutions	12.4 – 13.0	8.5 – 9.9 (84 – 98)	8.2 – 8.6
Old Solutions	11.7 – 12.9	3.6 – 11.5 (35 – 112)	7.5 – 8.5

Notes: Beginning at Day 1, gentle aeration was provided on all new solutions after renewals at a rate of 60-100 bubbles/minute. After aeration was provided, dissolved oxygen remained ≥4.0 mg/L (40% saturation) for the remainder of the study.

100% saturation at 12 and 13°C corrected for local altitude and mean barometric pressure is 10.3 and 10.1 mg/L, respectively.

Statistical Analysis:

Rainbow Trout 7-Day Static Renewal Test Statistical Analysis (mg/L)						
Sample ID	7-Day Survival			7-Day Growth		
	LC_{50} (95% CI)	NOEC	LOEC	EC_{50} (95% CI)	NOEC	LOEC
EK-35	23 (19 27)	10	20	>10	10	>10

Discussion:

Solution Preparation:

Solutions were prepared on a weight to volume basis for both compounds. EK-35 and EnviroKleen were not water-soluble and were conducted as the water accommodated fraction (WAF). EK-35 was weighed out on glass microscope slides and added to the preparation jars of water. The jars were placed on a stir plate and stirred overnight. Solutions were drawn off by siphoning the solutions into replicate test chambers. This undissolved test compound was not included in solutions for two reasons. One, so that it would not cause a decrease in dissolved oxygen transfer by covering the surface of the test vessels, and two, so that it would not cause secondary toxicity from impairment of the test fish respiratory system in the case of the rainbow trout gills.

Test Design:

A 72-hour static range-finding study was conducted at nominal loading concentrations of 0 (control), 10, 100, and 1,000 mg/L. Mortality was 0, 0, 100, and 100% in the EK-35 concentrations of 0 (control), 10, 100, and 1,000 mg/L, respectively. No mortality was observed in the any of the EnviroKleen concentrations. Based upon the results of the range-finding test, the definitive tests were conducted at nominal loading concentrations of 0 (control), 5.0, 10, 20, 40, and 80 mg/L for EK-35

Statistical Analysis:

Statistical analysis of the concentration versus effect data was performed using a custom computer program, ToxCalc. This program is designed to calculate the LC_{50}/EC_{50} statistic and its 95% confidence interval (CI), where possible, using the appropriate EPA recommended analysis. Statistical significance of comparison of means for Rainbow Trout survival and growth was determined by hypothesis testing using EPA recommended methods, typically either Fisher's Exact test or Dunnett's test. Point estimates testing to calculate the LC_{50} or EC_{50} was determined with the Trimmed Spearman-Karber method where possible.

Generally, the statistical approach was as follows. Analysis of each endpoint between samples was evaluated by first analyzing the data for normality and homogeneity of variance with Shapiro-Wilk's Test and Kolmogorov D's Test before comparison of means. If the data were normally distributed and the variances were homogeneous, then analysis of variance (ANOVA) was utilized for the weight data along with Fisher's Exact Test or Dunnett's procedure for comparing the means. Survival data were analyzed using Fisher's Exact test and growth was analyzed using Dunnett's. If the assumptions of normality or homogeneity of variance were not met, transformations of the survival data were employed to allow the use of parametric procedures. If transformations (e.g., arcsine-square root transformation) of the survival data still did not meet assumptions of

normality and homogeneity, then the non-parametric test, Steel's Many-One Rank Test, was used to analyze these data.

Biological Results:

EK-35:

This study was conducted as a multi-concentration test with levels of 0 (control), 5.0, 10, 20, 40, and 80 mg/L. Mortality was 0% in the control. After seven days, mortality was 0, 0, 35, 95, and 100% in the 5.0, 10, 20, 40, and 80 mg/L, respectively. The 7-day LC_{50} for survival was 23 mg/L with 95% confidence intervals of 20 to 27 mg/L. For survival the no-observed effect concentration, or NOEC was 10 mg/L and the lowest observed effective concentration, or LOEC, was 20 mg/L. The 7-day EC_{50} for growth was 23 mg/L with 95% confidence intervals of 19 to 27 mg/L. For growth, the NOEC was 10 mg/L and the LOEC was >10 mg/L.