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TRADE SECRET*Study Title*

H-25767: Static, Acute, 96-Hour Screening Test with
Rainbow Trout, *Oncorhynchus mykiss*

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STUDY COMPLETED ON: July 1, 2003

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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Elkton Road, P.O. Box 50
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WORK REQUEST NUMBER: [REDACTED]

STUDY CODE NUMBER: [REDACTED]

Company Sanitized. Does not contain TSCA CBI

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Work Completed by:

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1 July 2003

Date

Issued by Study Director:

Robert A. Hoke

Robert A. Hoke, Ph.D.
Senior Research Ecotoxicologist and Manager

1 July 2003

Date

Company Sanitized. Does not contain TSCA CBI

STUDY INFORMATION

Substance Tested: [REDACTED]

Synonyms/Codes: • H-25767
[REDACTED]

Haskell Number: 25767

Composition: [REDACTED]

Known Impurities: [REDACTED]

Physical Characteristics: White liquid

Stability: The test substance appeared to be stable under the conditions of the study.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: May 12, 2003 / (see report cover page)

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SUMMARY

The acute toxicity of H-25767 to the rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, 96-hour, static test.

The study was conducted with 4 concentrations of H-25767 and a dilution water control at a mean temperature of 12.8°C (range of 12.7-13.0°C). One test chamber was used per test concentration with 10 test organisms in each chamber. Based on visual observations, the dilution water control and 1 and 10 mg/L test concentration solutions were clear and colorless at test start. The 100 mg/L test concentration solution was slightly cloudy with a surface film at test start. The 1000 mg/L test concentration solution was cloudy with a surface film at test start. All water quality parameters were within acceptable limits during the exposure.

Exposure of rainbow trout to the dilution water control and nominal H-25767 concentrations of 1, 10, 100, and 1000 mg/L resulted in 0, 0, 0, 0, and 100% mortality, respectively, at the end of 96 hours. No mortality or sublethal effects were seen in the dilution water control test organisms. The highest nominal concentration causing no mortality at test end was 100 mg/L. The lowest nominal concentration causing 100% mortality at test end was 1000 mg/L. Nominal H-25767 concentrations were used for calculation of LC₅₀ values.

The results are summarized as follows:

Nominal concentrations of H-25767, mg/L	dilution water control, 1, 10, 100, and 1000
96-hour LC ₅₀ (95% confidence interval) for H-25767, based on nominal concentrations, mg/L ^a	316 (100 to 1000)

H-25767 exhibited low concern for aquatic hazard^b in an unaerated, 96-hour, static acute test using the rainbow trout, *Oncorhynchus mykiss*.

- a Stephan, C.E. (1997). Methods for calculating an LC₅₀. *Aquatic Toxicology and Hazard Evaluation, Proceedings of the First Annual Symposium on Aquatic Toxicology, ASTM Special Publication 634* (F.L. Mayer and J.L. Hamelink, Eds.), pp 65-84. Philadelphia.
- b Smrcek, J., Clements, R., Morcock, R., and Rabert, W. (1993). Assessing ecological hazard under TSCA: methods and evaluation of data. *Environmental Toxicology and Risk Assessment, ASTM STP 1179*. (W.G. Landis, J.S. Hughes, and M.A. Lewis, Eds.), pp 22-39. American Society for Testing and Materials, Philadelphia.

MATERIALS AND METHODS

A. Test Solution Preparation

Test substance solutions were prepared by direct addition of H-25767 to dilution water. The nominal concentrations of test substance solutions were not adjusted for purity. Test solutions were prepared by adding the appropriate amount of test substance to each test chamber which contained 10 L of Haskell Laboratory well water and stirring for approximately 44 minutes. The dilution water control and 1 and 10 mg/L test concentration solutions were clear and colorless at test start. The 100 mg/L test concentration solution was slightly cloudy with a surface film at test start. The 1000 mg/L test concentration solution was cloudy with a surface film at test start.

B. Dilution Water

Dilution water originated from the Haskell Laboratory well which is 378-feet deep and is cased and sealed to bedrock. The hardness of the well water is adjusted to approximately 100-140 mg/L as CaCO_3 by the flow-proportioned addition of CaCl_2 . The well water is then aerated, passed through a green sand filter to remove iron, and filtered through 50-, 10-, and 0.45- μm filters to remove particulates. The water is heated or chilled as appropriate and distributed through aged polyvinyl chloride piping. The dilution water is analyzed twice yearly for major anions and cations, metals, total organochlorine and organophosphate pesticides, and polychlorinated biphenyls. The dilution water meets OECD⁽¹⁾ and ASTM⁽²⁾ criteria and specifications.

C. Test Organism Culture

Rainbow trout, *Oncorhynchus mykiss*, were purchased from Thomas Fish Company, Anderson, California. The fish were held in a 272-L, circular, fiberglass holding tank (84-cm diameter, 49-cm depth) for 18 days in continuously-flowing well water at Haskell Laboratory. During the 7-day period preceding the test, water temperatures ranged from 10.4 to 11.2°C (mean 10.9°C). The fish were fed freshly-hatched, live brine shrimp (San Francisco Bay Brand, Inc.) and AquaMax® Starter Fingerling 300 5D03 (PMI® Nutrition International, LLC) twice daily on weekdays and once daily on weekends and holidays. Sickness, injury, and visible abnormalities were not present in fish in the holding tank nor in the fish used for testing. Mortality was <1% during the 48 hours prior to use for testing. Fish were identified by labels on the holding tanks and test chambers.

D. Test Methods

Four nominal test concentrations and a dilution water control were used in this study. The nominal test concentrations were 1, 10, 100, and 1000 mg/L H-25767.

Test chambers were stainless steel aquaria [30 (length) x 30 (width) x 30 (height) cm] which held approximately 10 L of test solution (26-L maximum volume; 11.5-cm test solution depth). One test chamber was used per test solution (total of 5 test chambers; 10 fish in each test chamber). Each chamber was covered with a glass plate to prevent the fish from escaping.

Addition of fish to the test solutions was initiated after mixing of the test solutions was completed. Mortality and behavioral observations were made every 24 hours and at test end. Dead fish were removed from the test chambers during daily observations. Criteria for death were the absence of opercular movement and lack of reaction to gentle prodding. At test conclusion, all fish were humanely sacrificed using MS222 (3-aminobenzoic acid ethyl ester).

A recirculating water bath was used to maintain mean temperature in the test chambers during the 96-hour test at approximately 12.8°C with a range of 12.7 to 13.0°C. A photoperiod of 16 hours light (approximately 223 to 484 Lux) and 8 hours darkness was employed which included 30 minutes of transitional light (62 to 198 Lux) preceding and following the 16-hour light interval.

Dissolved oxygen concentration, pH, and temperature were measured in all test chambers. These measurements were taken before fish were added at test start, every 24 hours thereafter, and at test end. Test solutions were not aerated during the test and were disposed of in an appropriate manner at test end.

RESULTS AND DISCUSSION

Exposure of rainbow trout to the water control and nominal H-25767 concentrations of 1, 10, 100, and 1000 mg/L resulted in 0, 0, 0, 0, and 100% mortality, respectively, at the end of 96 hours. No mortality or sublethal effects were seen in the dilution water control test organisms. The highest nominal concentration causing no mortality at test end was 100 mg/L. The lowest nominal concentration causing 100% mortality at test end was 1000 mg/L. Nominal H-25767 concentrations were used for calculation of LC₅₀ values.

The 96-hour LC₅₀ for H-25767 to rainbow trout was 316 mg/L.

REFERENCES

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for the Testing of Chemicals: 203, 17 July 1992.
2. American Society for Testing and Materials (ASTM). (1988). Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E 729-88a. Annual Book of ASTM Standards, Vol. 11.04.

TABLE 1

DISSOLVED OXYGEN AND pH OF TEST AND CONTROL SOLUTION SAMPLES

Nominal H-25767 Concentration (mg/L)	Test Initiation (0 hours)		Test Completion (96 hours)	
	Dissolved Oxygen (mg/L)	pH	Dissolved Oxygen (mg/L)	pH
Water Control	10.7	7.7	9.1	7.3
1.0	10.7	7.7	8.9	7.3
10	10.7	7.7	9.0	7.3
100	10.7	7.7	8.8	7.3
1000	10.8	7.7	10.2 ^a	7.4 ^a

a Measurements taken at 24 hours due to total mortality in a test concentration.

TABLE 2

MORTALITY OF *Oncorhynchus mykiss* IN AN UNAERATED, STATIC, ACUTE, 96-HOUR
TEST WITH H-25767

Nominal H-25767 Concentration (mg/L)	Number Dead / Number at Test Initiation			
	24 Hours	48 Hours	72 Hours	96 Hours
Water Control	0/10	0/10	0/10	0/10
1.0	0/10	0/10	0/10	0/10
10	0/10	0/10	0/10	0/10
100	0/10	0/10	0/10	0/10
1000	10/10	10/10	10/10	10/10

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