

TRADE SECRET

Study Title

H-24750: Static, Acute, 96-Hour Screening Test to
Oncorhynchus mykiss

Laboratory Project ID: DuPont-12988

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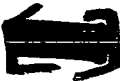
STUDY COMPLETED ON: May 14, 2003

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
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WORK REQUEST NUMBER:



STUDY CODE NUMBER:



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CERTIFICATION

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

Work Completed by: Laurie D. Bouchelle 14 May 2003
Laurie D. Bouchelle
Associate Scientist
Date

Issued by Study Director: Robert A. Hoke 14 May 2003
Robert A. Hoke, Ph.D.
Senior Research Ecotoxicologist and Manager
Date

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STUDY INFORMATION

9th Collective Nomenclature: [REDACTED]

Synonyms/Codes: [REDACTED]

H-24750

Submitter's Notebook Number(s): [REDACTED]

Haskell Number: 24750

CAS Registry Number: [REDACTED]

Purity: [REDACTED]

Known Impurities: [REDACTED]

Physical Characteristics: White Solid

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

Study Initiated/Completed: April 14, 2003 / (see report cover page)

In-Life Initiated/Completed: April 14, 2003 / April 18, 2003

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SUMMARY

The acute toxicity of H-24750 to the rainbow trout, *Oncorhynchus mykiss* was determined in an unaerated, 96-hour, non-GLP, static test.

The study was conducted with 5 concentrations of H-24750 and a dilution water control at a mean temperature of 11.7°C. One test chamber was used per test concentration with 10 test organisms in each chamber. Based on visual observations, the dilution water control, 0.1 and 1.0 mg/L test concentrations were clear with no color at test start. The 10 mg/L test concentration was clear and colorless with undissolved test material present on the bottom of the test chamber. The 100 mg/L test concentration was clear and colorless with a surface film and undissolved test material present on the bottom of the test chamber. The 1000 mg/L test concentration was cloudy with a surface film and undissolved test material present on the bottom of the test chamber. All water quality parameters were within acceptable limits during the exposure with the exception of the pH (3.9 – 4.2) in the 1000 mg/L test concentration.

Exposure of rainbow trout to the dilution water control and nominal H-24750 concentrations of 0.10, 1.0, 10, 100, and 1000 mg/L resulted in 0, 0, 0, 0, 100, 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 10 mg/L. The lowest nominal concentration causing 100% mortality at test end was 100 mg/L. Nominal H-24750 concentrations were used for calculation of LC₅₀ values.

The results are summarized as follows:

Nominal concentrations of H-24750, mg/L	dilution water control, 0.10, 1.0, 10, 100 and 1000
96-hour LC ₅₀ (95% confidence interval) for H-24750, based on nominal concentrations, mg/L ^a	32 (10 to 100)

H-24750 exhibited medium 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-24750 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 15 L of Haskell Laboratory well water and stirring for approximately 45 minutes. The water control, 0.1 and 1.0 mg/L test concentrations were clear with no color at test start. The 10 mg/L test concentration was clear and colorless with undissolved test material present on the bottom of the test chamber. The 100 mg/L test concentration was clear and colorless with a surface film and undissolved test material present on the bottom of the test chamber. The 1000 mg/L test concentration was cloudy with a surface film and undissolved test material present on the bottom of the test chamber.

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 received as unfertilized eggs and sperm from Troutlodge, Inc., Sumner, Washington. The eggs were fertilized in the laboratory using the sperm received with the eggs. The fish were held in a 272-L, circular, fiberglass holding tank (84-cm diameter, 49-cm depth) for 28-34 days after hatching in continuously-flowing well water at Haskell Laboratory. During the 7-day period preceding the test, water temperatures ranged from 12.0 to 12.1°C (mean 12.1°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. Less than 0.5% mortality of fish occurred in the holding tank during the 48 hours prior to use for testing. Fish were identified by labels on the holding tanks and test chambers.

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D. Test Methods

Five nominal test concentrations and a dilution water control were used in this study. The nominal test concentrations were 0.10, 1.0, 10, 100, and 1000 mg/L H-24750.

Test chambers were stainless steel aquaria [30 (length) x 30 (width) x 30 (height) cm] which held approximately 15 L of test solution (26-L maximum volume; 17.5-cm test solution depth). One test chamber was used per test solution (total of 6 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 11.7°C. A photoperiod of 16 hours light (approximately 264 to 424 Lux) and 8 hours darkness was employed which included 30 minutes of transitional light (11 to 37 Lux) preceding and following the 16-hour light interval.

Dissolved oxygen concentration, pH, and temperature were measured in all test chambers (Table 1). 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

The study was conducted with 5 concentrations of H-24750 and a dilution water control at a mean temperature of 11.7°C. One test chamber was used per test concentration with 10 test organisms in each chamber. Based on visual observations, the dilution water control, 0.1, and 1.0 mg/L test concentrations were clear with no color at test start. The 10 mg/L test concentration was clear and colorless with undissolved test material present on the bottom of the test chamber. The 100 mg/L test concentration was clear and colorless with a surface film and undissolved test material present on the bottom of the test chamber. The 1000 mg/L test concentration was cloudy with a surface film and undissolved test material present on the bottom of the test chamber. All water quality parameters were within acceptable limits during the exposure with the exception of the pH (3.9 – 4.2) in the 1000 mg/L test concentration.

Exposure of rainbow trout to the dilution water control and nominal H-24750 concentrations of 0.10, 1.0, 10, 100, and 1000 mg/L resulted in 0, 0, 0, 0, 100, and 100% mortality, respectively, at the end of 96 hours (Table 2). 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 10 mg/L. The lowest nominal concentration causing 100% mortality at test end was 100 mg/L. Nominal H-24750 concentrations were used for calculation of LC₅₀ values.

Toxicity data analysis was conducted based on nominal test concentrations using the binomial method. The 96-hour LC₅₀ for H-24750, based on nominal concentrations, was 32 mg/L, with a 95% confidence interval of 10 to 100 mg/L. H-24750 exhibited medium concern for aquatic hazard^c in an unaerated, 96-hour, static, acute test using the rainbow trout, *Oncorhynchus mykiss*.

REFERENCES

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for Testing 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.

^c 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.

TABLE 1

DISSOLVED OXYGEN AND pH OF TEST AND CONTROL SOLUTION SAMPLES

Nominal H-24750 Concentration (mg/L)	Test Initiation (0 hours)		Test Completion (96 hours)	
	Dissolved Oxygen (mg/L)	pH	Dissolved Oxygen (mg/L)	pH
Water Control	10.8	7.6	8.9	7.3
0.10	10.9	7.6	9.1	7.3
1.0	10.5	7.5	9.0	7.3
10	10.3	7.5	8.9	7.3
100	10.3	7.2	9.6 ^a	7.2 ^a
1000	10.1	4.2	10.3 ^b	3.9 ^b

a Measurement performed at 48 hours due to total mortality.

b Measurement performed at 24 hours due to total mortality.

TABLE 2

MORTALITY OF *Oncorhynchus mykiss* IN AN UNAERATED, STATIC, ACUTE,
96-HOUR TEST WITH H-24750

Nominal H-24750 Concentration (mg/L)	Number of Dead Test Organisms / Number of Test Organisms at Study Start			
	24 Hours	48 Hours	72 Hours	96 Hours
Water Control	0/10	0/10	0/10	0/10
0.10	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	8/10	10/10	10/10	10/10
1000	10/10	10/10	10/10	10/10

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