

TRADE SECRET

Study Title

H-24750: Static, Acute, 48-Hour Toxicity Screening Test with *Daphnia magna*

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SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
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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:

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14 January 2004

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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: 99.6%

Known Impurities: Not supplied by the sponsor

Physical Characteristics: White Solid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: December 29, 2003 / (see report cover page)

In-Life Initiated/Completed: December 29, 2003 / December 31, 2003

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SUMMARY

The acute toxicity of H-24750 to the water flea, *Daphnia magna* (less than 24 hours old) was determined in an unaerated, 48-hour, static test.

The study was conducted with 4 concentrations of H-24750 and a dilution water control at a mean temperature of 20.4°C (range of 20.0 - 20.8°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 0.10, 1.0, and 10 mg/L test concentrations were clear and colorless with no undissolved test material at test start. The 100 mg/L test concentration was slightly cloudy with undissolved test material settled on the bottom of the test chamber at test start. All water quality parameters were within acceptable limits during the exposure.

Exposure of daphnids to the dilution water control and nominal H-24750 concentrations of 0.10, 1.0, 10, and 100 mg/L resulted in 0, 0, 0, 10, and 20% immobility, respectively, at the end of 48 hours. No immobility or sublethal effects were seen in the dilution water control test organisms. The highest nominal concentration causing no immobility at test end was 1.0 mg/L. The lowest nominal concentration causing 100% immobility at test end was greater than 100 mg/L.

The results are summarized as follows:

Nominal concentrations of H-24750, mg/L	dilution water control, 0.10, 1.0, 10, and 100
48-hour EC ₅₀ for H-24750, based on nominal concentrations and immobility, mg/L	greater than 100

H-24750 exhibited low concern for aquatic hazard⁽¹⁾ in an unaerated, 48-hour, static acute test using the water flea, *Daphnia magna* (less than 24 hours old).

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MATERIALS AND METHODS

A. Test Solution Preparation

Test solutions of 1.0, 10, and 100 mg/L were prepared by adding the appropriate amount of test material to Haskell Laboratory Well Water (HLWW) in 1-L beakers, stirring for approximately 5 minutes, sonicating for approximately 45 minutes, and stirring for approximately 5 minutes. The 0.10 mg/L test solution was then prepared by adding 100 mL of the 1.0 mg/L test solution to 900 mL HLWW in a 1-L beaker. After the preparation of the 0.01 mg/L test solution, all test solutions and the dilution water control were stirred for an additional 15 minutes. The dilution water control and 0.10, 1.0, and 10 mg/L test concentrations were clear and colorless with no undissolved test material at test start. The 100 mg/L test solution was slightly cloudy with undissolved test material settled on the bottom of the test vessel 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 HLWW is adjusted to approximately 100-140 mg/L as CaCO_3 by the flow-proportioned addition of CaCl_2 . The HLWW is then aerated, passed through a green sand filter to remove iron, and filtered through 50-, 10-, and 3- 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⁽²⁾ and ASTM⁽³⁾ specifications.

C. Test Organism Culture

Daphnia magna were reared at Haskell Laboratory in 1000-mL Pyrex[®] beakers (10 per beaker at culture initiation) which contained 1000 mL of aerated, filtered HLWW held at approximately 20.5°C. Daphnids were fed on a daily basis with 2 mL/L of a yeast, cereal leaves and trout chow (YCT) mixture (standardized to 1700 to 2100 mg/L total solids) and the green alga, *Selenastrum capricornutum*, at a rate of 62,500 cells/mL of culture medium. The combination of YCT and alga is equivalent to 0.1-0.2 mg total organic carbon per daphnid. Neonates used in this test were less than 24 hours old and were collected from the 4th brood of 12-day old parent daphnids. Sickness, injury, and abnormalities were not seen and ephippia were not being produced by the parent daphnids. No adult immobility was seen in the cultures used for testing during the 48-hour pretest period. *Daphnia magna* were identified by labels on the culture beakers and test chambers.

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D. Test Methods

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

Pyrex® beakers (250-mL) containing 200 mL of test solution (6.8-cm test solution depth) were used as test chambers. One replicate test chamber was used per test concentration with 10 daphnids in each chamber. The test chambers were covered with a glass plate during the test.

Daphnia magna neonates, less than 24 hours old, were used in this study. Daphnids were not fed during the test. Addition of daphnids to test solutions was initiated after mixing of the test solutions was completed. Immobility and behavioral observations were made daily. The criterion for the effect (immobility) was a lack of reaction to application of a gentle stimulus.

A recirculating water bath was used to maintain mean temperature in the test chambers during the 48-hour test at approximately 20.4°C with a range of 20.0 to 20.8°C. A photoperiod of 16 hours light (approximately 591 to 810 Lux) and 8 hours darkness was employed which included 30 minutes of transitional light (23 to 52 Lux) preceding and following the 16-hour light interval.

Dissolved oxygen concentration, pH, and temperature were measured in all replicates of the dilution water control and test substance concentrations. These measurements were taken before daphnids were added at test start, and at test end or at total immobility in a replicate chamber. Test solutions were not aerated during the test and were disposed of in an appropriate manner at test end.

RESULTS AND CONCLUSION

Exposure of daphnids to the dilution water control and nominal H-24750 concentrations of 0.10, 1.0, 10, and 100 mg/L resulted in 0, 0, 0, 10, and 20% immobility, respectively, at the end of 48 hours. No immobility or sublethal effects were seen in the dilution water control test organisms. The highest nominal concentration causing no immobility at test end was 1.0 mg/L. The lowest nominal concentration causing 100% immobility at test end was greater than 100 mg/L.

The 48-hour EC₅₀ for H-24750, based on nominal concentrations and immobility, was greater than 100 mg/L. H-24750 exhibited low concern for aquatic hazard⁽¹⁾ in an unaerated, 48-hour, static acute test using the water flea, *Daphnia magna* (less than 24 hours old).

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REFERENCES

1. 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.
2. Organisation for Economic Co-Operation and Development (OECD). Guideline for the Testing of Chemicals: 203, 17 July 1992.
3. 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.

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TABLE 1

DISSOLVED OXYGEN AND pH OF TEST AND
DILUTION WATER CONTROL SOLUTION SAMPLES

Nominal H-24750 Concentration (mg/L)	Test Initiation (0 hours)		Test Completion (48 hours)	
	Dissolved Oxygen (mg/L)	pH	Dissolved Oxygen (mg/L)	pH
Dilution Water Control	7.6	7.3	8.7	8.0
0.10	7.5	7.6	8.6	8.0
1.0	7.5	7.5	8.7	8.0
10	7.6	7.5	8.7	7.9
100	7.8	7.2	8.8	7.8

TABLE 2

IMMOBILITY AND SUBLETHAL EFFECTS OF *Daphnia magna* IN AN UNAERATED,
STATIC, ACUTE, 48-HOUR TEST WITH H-24750

Nominal H-24750 Concentration (mg/L)	Number Immobile (Number Affected*) / Number at Study Start	
	24 Hours	48 Hours
Dilution Water Control	0/10	0/10
0.10	0/10	0 ^{1a} /10
1.0	0/10	0 ^{3a} /10
10	0 ^{3a} /10	1 ^{4a} /10
100	0 ^{4a} /10	2 ^{2a} /10

* effects given in superscript

a daphnid lethargic

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