

CODING FORMS FOR SRC INDEXING

| | | | |
|--------------------------------|---|----------------------|-------------|
| Microfiche No. | | OTS0001066 | |
| New Doc ID | FYI-OTS-0794-1066 | Old Doc ID | 84940000158 |
| Date Produced | 04/26/84 | Date Received | 07/14/94 |
| | | TSCA Section | FYI |
| Submitting Organization | MONSANTO CO | | |
| Contractor | | | |
| Document Title | INITIAL SUBMISSION: 48-HOUR DAPHNIA MAGNA ACUTE TOXICITY STUDY f | | |
| Chemical Category | HEXAMETHYLENE DIAMINE | | |

7/11-0794-000066



FBI-94-681866
INIT 07/14/94



84948888158

Monsanto

DEPARTMENT OF MEDICINE &
ENVIRONMENTAL HEALTH

Monsanto Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167
Phone: (314) 694-1000

February 8, 1985

Contains No CBI

Mr. Lou Borghi
Dyna-Mac Corporation
11140 Rockville Pike
Rockville, Maryland 20852

Dear Mr. Borghi:

As requested I am forwarding a copy of the 48 hour Daphnia magna acute toxicity study with heaxmethylen diamine.

Sincerely,

James W. Barnett, PhD
Product Toxicology Specialist

JWB/pp
Enclosure

RECEIVED
OPPT/CBIC
94 JUL 14 AM 9:23

INTRODUCTION

The purpose of this study is to determine the acute toxicity of hexamethylene-diamine to a common aquatic invertebrate Daphnia magna. The use of Daphnia magna as a representative aquatic species is viewed by the scientific community (Cairns et al., 1979) as a valid means of obtaining an estimate of the toxicity of a chemical to aquatic organisms. Toxicological information together with an estimate of environmental exposure concentration can be used to evaluate the potential environmental hazard of chemical resulting from manufacture, use, and disposal.

SUMMARY

The acute toxicity of hexamethylene diamine to Daphnia magna was assessed at the aquatic laboratory during a 48-hour static test. The 48-hour EC50 value is 50 mg/l and the no effect level was observed to be 32 mg/l.

MATERIALS AND METHODS

Procedures used in the acute toxicity test closely followed those described in the MIC Environmental Assessment Method for Conducting Acute Toxicity Tests with Daphnia magna (Grueber and Adams, 1980), and Methods for Acute Toxicity Tests With Fish, Macroinvertebrates and Amphibians (U.S. EPA, 1975). The Daphnia magna used in this test were cultered at the MIC aquatic laboratory and came from adults which were fed a trout chow solution and algae daily. Daphnids known to be less than 24 hours old were separated from the adults and used for this test. Nominal concentrations are reported as milligrams of test compound per liter of dilution water (mg/L). The chemical used in this test was obtained from John Roberts, at Monsanto's Greenwood, S.C. production facility. All raw data pertaining to this study is contained in Appendix I and Monsanto notebook page 2033500. The test chemical, hexamethylenediamine, was received in a 500 ml. nalgene jar in good condition on 10 April 1984. The chemical has been labeled and logged in Monsanto notebook on page 2764501 and is stored in the Environmental Assessment Group chemical storage cabinet. The test was started and finished on 11 April and 13 April, 1984.

The static toxicity test was conducted in 250 milliliter (mL) beakers which contained 200 mL of test solution. The dilution water used in this study was well water from St. Peters, Missouri. For each test concentration, the appropriate amount of the test compound was pipetted into 1000 mL of dilution water and shaken vigorously for 1 minute. This solution was then divided into four 200 mL aliquots in four beakers to provide appropriate replication. The remaining 400 mL were used for 0-hour DO, pH, alkalinity and hardness determinations. A control, consisting of the same dilution water and conditions but with no test compound was established. No solvent was necessary as the test chemical is water soluble.

Nominal test concentrations were selected based on a rangefinding test (Appendix I). All test vessels were maintained at room temperature. Test solutions were not aerated during the test. Five daphnids were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 20 daphnids per concentration.

During this test, the dissolved oxygen concentration, pH alkalinity and hardness, and temperature of test solutions were monitored at the initiation and termination of the toxicity test in the high, middle, low and control test concentrations. DO was measured with a Beckman pH meter (SOP #EAS-80-SOP-007). The total hardness and alkalinity determinations were conducted according to "Standard Methods for the Examination of Water and Wastewater" (1979), (SOPs is presented in Table 1. All raw data for Table I is contained on notebook pages 1,889,925-1,889,927.

Test concentrations and corresponding percent mortality data derived from definitive tests were used to calculate the 48-hour median effect concentration, EC50, and 95% confidence intervals. The EC50 is defined as the calculated nominal concentration of the test compound in dilution water which causes 50% immobilization in the test animal population at the stated exposure interval.

In tests where the highest percentage was ≥ 65 percent, the computer program of Stephen (1978) which calculates an EC50 by three methods, binomial, moving average, and probit analysis, was used (Stephen, 1976). For tests in which the mortality did not exceed 50 percent, the EC50 is reported as greater than the highest test concentration. If the highest percentage kill was >50 <65 percent, the EC50 is estimated by the program of Stephen and is reported as an estimate.

RESULTS

During the 48-hour toxicity test with hexamethylenediamine, the pH and dissolved oxygen ranged from 7.6 to 8.8 and 8.6 to 9.0 mg/L, respectively (Table 2 and Appendix I). The average temperature was 20.0°C and the alkalinity and hardness ranged from 204 to 234 mg/L and 208 to 234 mg/L. A summary of the percent immobilization during this test is presented in Table 3. The 24 and 48 hour Lc50 values were 80 mg/l and 50 mg/l, respectively (Table 4).

QUALITY ASSURANCE

All aspects of this study meet the recommended criteria for an acceptable test as specified in Grueber and Adams (1980). Both a control and a solvent control were used in this study. Good laboratory procedures were followed as described in the Monsanto Research Corporation Good Laboratory Practices Manual (1983).

TABLE 1. Average water quality characteristics of the dilution (well) water.

| Characteristic | Well Water Measurement |
|---|------------------------|
| Alkalinity (mg/l CaCO ₃) | 303 |
| Hardness (mg/l CaCO ₃) | 297 |
| pH (median) | 8.10 |
| Aluminum (mg/l Al) | 0.014 |
| Ammonia-total (mg/l N) | 0.35 |
| Ammonia-unionized (mg/l NH ₃) | 0.001 |
| Antimony (mg/l Sb) | 0.006 |
| Barium (mg/l B) | <0.035 |
| Beryllium (mg/l Be) | 0.001 |
| Cadmium (mg/l Cd) | <0.001 |
| Calcium (mg/l Ca) | 67.0 |
| Chloride (mg/l Cl ⁻) | 54.5 |
| Chromium (mg/l Cr) | <0.009 |
| Cobalt (mg/l Co) | 0.002 |
| Copper (mg/l Cu) | 0.005 |
| Fluoride (mg/l F ⁻) | 1.90 |
| Iron (mg/l Fe) | 0.013 |
| Lead (mg/l Pb) | 0.01 |
| Magnesium (mg/l Mg) | 2.79 |
| Manganese (mg/l Mn) | 0.001 |
| Molybdenum (mg/l Mo) | 0.005 |

TABLE 1. cont'd.

| Characteristic | Well Water Measurement |
|---|------------------------|
| Nickel (mg/l Ni) | 0.025 |
| Phosphorus (mg/l P) | 0.006 |
| Silicon (mg/l Si) | 4.3 |
| Silver (mg/l Ag) | <0.001 |
| Sodium (mg/l Na) | 83.2 |
| Sulfate (mg/l SO ₄ ⁻²) | 175.4 |
| Strontium (mg/l Sr) | 1.30 |
| Tin (mg/l Sn) | 0.006 |
| Titanium (mg/l Ti) | 0.001 |
| Total Organochlorine (ug/l) | <0.5 |
| Total Organophosphorus (ug/l) | <0.05 |
| Vanadium (mg/l V) | 0.14 |
| Zinc (mg/l Zn) | 0.006 |

Table 2. Temperature, Dissolved Oxygen Concentrations, pH, Alkalinity, and Hardness Measurements Taken During the 48-Hour Acute Test With Hexamethylenediamine and Daphnia magna.

| Measurement | Conc. (mg/l) | 0-Hour | 48-Hour |
|-------------------|--------------|--------|---------|
| Temperature (°C) | Control | 20.1 | 20.6 |
| | 4 | 19.7 | 20.4 |
| | 36 | 19.7 | 20.4 |
| | 100 | 19.3 | 20.3 |
| D.O. (mg/L) | Control | 8.8 | 8.8 |
| | 4 | 8.9 | 8.9 |
| | 36 | 9.0 | 8.8 |
| | 100 | 8.8 | 8.6 |
| pH | Control | 7.6 | 8.1 |
| | 4 | 7.9 | 8.2 |
| | 36 | 8.5 | 8.2 |
| | 100 | 8.8 | 8.3 |
| Alkalinity (mg/L) | Control | 216 | 206 |
| | 4 | 210 | 204 |
| | 36 | 218 | 220 |
| | 100 | 228 | 234 |
| Hardness (mg/L) | Control | 228 | 220 |
| | 4 | 208 | 210 |
| | 36 | 220 | 226 |
| | 100 | 228 | 234 |

Table 3. Concentrations tested and corresponding percent immobilization of Daphnia magna exposed to hexamethylenediamine.

| Nominal Concentration (mg/l) | Percent Immobilization for Combined Replicates | |
|------------------------------------|--|----------|
| | 24 Hours | 48 Hours |
| Control | 0 | 5 |
| 3.5 | 0 | 0 |
| 7 | 0 | 0 |
| 11 | 0 | 0 |
| 18 | 0 | 0 |
| 32 | 0 | 0 |
| 53 | 0 | 5 |
| 88 | 65 | 55 |
| | | 100 |

Table 4. Acute toxicity of hexamethylenedfamine. to Japinia magna.

| EC50 Values mg/L (95% C.I.) | | No Effect Concentration at 49 Hours (mg/L) |
|-----------------------------|---------------|--|
| 24 Hours | 48 Hours | |
| 80 (53 - ∞) | 50 (44-56) | 32 |

LITERATURE CITED

- American Society for Testing Materials. 1980. Standard Practice for Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates, and Amphibians. ASTM Standard Practice Designation: E729-80.
- Cairns, J. Jr., K.L. Dickson and A.W. Maki, 1978. Estimating the Hazard of Chemical Substances to Aquatic Life. American Society for Testing Materials. ST: 657.
- Grueber, D.J. and W. J. Adams, 1980. MIC Environmental Assessment Method for Conducting Acute Tests With Daphnia magna. Environmental Sciences Report ES-80-M-6.
- Standard Methods for the Examination of Water and Wastewater. 1971. 13th Edition, New York, 974.
- Standard Operating Procedure MIC Environmental Sciences. Dissolved Oxygen Determination - Winkler Method. 1980. Document number EAS-80-SOP-006.
- Standard Operating Procedure MIC Environmental Sciences. pH Determination. 1980. Document number EAS-80-SOP-007.
- Standard Operating Procedure MIC Environmental Sciences. Alkalinity Measurement. 1980. Document number EAS-80-SOP-008.
- Standard Operating Procedure MIC Environmental Sciences. Hardness Determination in Test and Culture Water. 1980. Document number EAS-80-SOP-009.
- Stephan, C.E. 1976. Methods for Calculating an LC50. In Aquatic Toxicology and Hazard Evaluation, F. L. Mayer and J. L. Hamelink Editors, ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA, pp 65-84.
- Stephan, C.E. 1978. Personal communication. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, MN.
- U.S. EPA 1975. Methods for Acute Toxicity Tests With Fish, Macroinvertebrates and Amphibians. Ecological Research Series, EPA 660/3-75-009, 61 pp.
- Monsanto Research Corporation. 1983. Good Laboratory Practices Manual. Monsanto Company, Environmental Sciences Center, St. Louis, MO.

Submitted by:

Monsanto Company
Environmental Sciences Section - N1B
800 North Lindbergh Boulevard
St. Louis, Missouri 63167

Prepared by:

P. Scott Zilgenfuss

William J. Adams

Approved by:

Richard Kemule

APPENDIX I

-Raw Data-

MIC ENVIRONMENTAL SCIENCES REPORT NO.

TYPE OF TEST: Rangfinder
 TEST SPECIES: Streaked Gnatcatcher
 TEST MATERIAL: Encephalium magna
 FORM ADMINISTERED: Heard's LOT NO: NB9 2764501
 STOCK CONC: 100 ml/l (ppm)
 TEST CONTAINER VOLUME: 250 TEST SOLUTION VOLUME: 200
 COMMENTS: CD

DATE TEST STARTED: 11 April 84
 AGE: 524 dpa
 DATE RECEIVED: 10 April 84
 CULTURE # NA

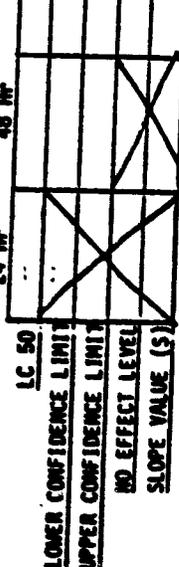
DATE TEST ENDED: 13 April 1984
 DIRECTED BY: Scott Ziegenfuss
 TIME TEST STARTED: 12:00 noon
 PHYSICAL STATE: clear liquid

NO. OF ANIMALS/CONC.: 10
 NO. OF CONC.: 4 + Control

TEST RESULTS

| TIME (HOURS) | NO. OF MORTALITIES AT EACH CONC. (PPM) | | | | | CHEMICAL MEASUREMENTS AT EACH CONC. () | | | | | |
|-----------------|--|-----|----|---|-----|---|----------|----------------------|----------|-----|--------------|
| | 0 | .04 | .4 | 4 | 100 | TIME | CON. 100 | DISSOLVED OXYGEN ppm | CON. 100 | PH | OBSERVED BY: |
| 0 | 1 | 1 | 1 | 1 | 1 | 0 | 70 | 6.9 | 7.5 | 9.1 | PSZ |
| 24 | 1 | 1 | 1 | 1 | 1 | 24 | 68 | 7.0 | 8.1 | 8.5 | PSZ |
| 48 | 1 | 1 | 1 | 1 | 1 | 48 | | | | | |
| 72 | 1 | 1 | 1 | 1 | 1 | 72 | | | | | |
| 96 | 1 | 1 | 1 | 1 | 1 | 96 | | | | | |
| TOTAL MORTALITY | 1 | 1 | 1 | 1 | 1 | | | | | | |

| TIME | ALKALINITY | | HARDNESS | | TEMPERATURE | |
|------|------------|-----|----------|-----|-------------|-----|
| | CON. | 100 | CON. | 100 | CON. | 100 |
| 0 | | | | | | |
| 24 | | | | | | |
| 48 | | | | | | |
| 72 | | | | | | |
| 96 | | | | | | |



| SPECIES | TEST | CHEMICAL | SOLVENT | NAME (INT) | DATE |
|---------------|----------------|----------------------|--------------|--------------------------|-----------------|
| DAPHNIA MAGNA | CHEMICAL | HEXAMETHYLENEDIAMINE | | NCNE | S ZIEGENFUSS 25 |
| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) | |
| 100 | 20 | 13 | 65 | 13.1563 | |
| 50 | 20 | 0 | 0 | 9.53675E-05 | |
| 25 | 20 | 0 | 0 | 9.53675E-05 | |
| 13 | 20 | 0 | 0 | 9.53675E-05 | |
| 8 | 20 | 0 | 0 | 9.53675E-05 | |
| 4 | 20 | 0 | 0 | 9.53675E-05 | |

THIS DATA SET DOES NOT MEET THE CRITERIA ESTABLISHED BY THE COMMITTEE ON METHODS FOR TOXICITY TEST WITH AQUATIC ORGANISMS BECAUSE NO PERCENT DEAD IS GREATER THAN 65 PERCENT.

THE BINOMIAL TEST SHOWS THAT 63 AND INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95% CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.9999 %.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS ~~55.4707~~ (u/l)

WHEN THERE ARE LESS THAN TWO CONCS. AT WHICH THE % DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AV. NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

WOULD YOU LIKE A PRINT OUT (YES=1, NO=0)?

| SPECIES | TEST | CHEMICAL | SOLVENT | NAME (INT) | DATE |
|---------|----------------|-------------|--------------|--------------------------|---------------|
| DAPHNIA | CHEMICAL | MAGNANONE | | S ZIEGENFUSS | 25 APRIL 1984 |
| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) | |
| 100 | 20 | 20 | 100 | 9.53675E-05 | |
| 50 | 20 | 11 | 55 | 41.1902 | |
| 25 | 20 | 0 | 0 | 9.53675E-03 | |
| 13 | 20 | 0 | 0 | 9.53675E-05 | |
| 8 | 20 | 0 | 0 | 9.53675E-05 | |
| 4 | 20 | 0 | 0 | 9.53675E-05 | |

THE BINOMIAL TEST SHOWS THAT 36 AND 100 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95% CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.9979 %.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 57.4707

| SPAN | G | LC50 | 95% CONFIDENCE LIMITS |
|------|-----------|---------|-----------------------|
| 1 | 051457 | 55.4707 | 48.6084 |
| 2 | 572925-02 | 57.4707 | 49.8103 |
| 1 | 292184 | | 79.0148 |

NO CONVERGENCE IN 25 ITERATIONS. THE PROBIT METHOD PROBABLY CANNOT BE USED WITH THIS SET OF DATA.

WOULD YOU LIKE A PRINT OUT (YES=1, NO=0)?

Determination of the Density
of Hexamethylene diamine (HMD)

As this chemical is a liquid, the density of this compound had to be determined in order to express the nominal concentrations in terms of mg/l (as opposed to ul/l.) This information could not be located in the standard references (CRC and Merch's Index), so the weight of 20.0 ml of the chemical was determined to calculate the density.

$$\begin{array}{rcl} \text{mass of beaker + 20 ml HMD} & = & 44.87265 \text{ g} \\ \text{mass of beaker} & = & 27.30534 \text{ g} \\ \text{mass of HMD (20.0 ml)} & = & \underline{17.56731 \text{ g}} \end{array}$$

$$\frac{17.56731 \text{ g}}{20.0 \text{ ml}} = \boxed{.878 \text{ g/ml}} = \text{density}$$

This density value was used to convert all concentration values in this report (LC50's, nominal concentrations, no effect concentration) to mg/l values.