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BN-82-276

DATA EVALUATION RECORD

1. CHEMICAL: Acetochlor
   Shaughnessey No: 121601

2. TEST MATERIAL: MON 097 technical; NBP 1992034; Lot #BA-29; 97.7% active ingredient; an amber colored liquid.

3. STUDY TYPE: Freshwater Fish Early Life Stage Test.
   Species Tested: Salmo gairdneri.

4. CITATION: Altshul, L. 1983. The Toxicity of MON 097 to Rainbow Trout Embryos and Larvae. Conducted by EG&G Bionomics Aquatic Toxicology Laboratory, Wareham, Massachusetts. Bionomics Report No. BW-83-4-1390. Submitted by Monsanto Chemical Company, St. Louis, Missouri. Monsanto Study No. BN-82-276.

5. REVIEWED BY:

   G. Scott Ward
   Manager
   Aquatic Toxicology Laboratory
   Toxikon Environmental Sciences

   Signature:

   Date:

6. APPROVED BY:

   Pim Kosalwat, Ph.D.
   Senior Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature:

   Date: Cynthia Moulton 10.30.90

   Henry T. Craven, M.S.
   Supervisor, EEB/HED
   USEPA

   Signature: Henry T. Craven

   Date: 10/30/90

7. CONCLUSIONS: The data submitted are not scientifically sound, and do not fulfill the guideline requirements for a freshwater fish early life stage test. The percent viability of solvent control and control embryos did not exceed 34%. Also, raw data were not submitted with the report for statistical verification. Based on the author's statistical analyses, no MON 097-related adverse effects were observed. Therefore, an MATC could not be determined from this study.

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8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The rainbow trout used in this test were received as separate portions of trout sperm and unfertilized eggs from Mount Lassen Trout Farm, Red Bluffs, California. Upon receipt of these gametes at EG&G Bionomics' laboratory, the sperm and eggs were gradually warmed to 12°C from the receipt temperature of 6°C, during a 1-hour period. After warming, sperm and eggs were mixed together to permit fertilization. Upon completion of water hardening, embryos were ready for distribution to the test system.

B. Test System: Trout exposures to MON 097 were conducted using a proportional diluter with a dilution factor of 0.5X. The diluter delivered the MON 097 test concentrations, the solvent control, and dilution water control volumes to duplicate glass aquaria (a total of 14) measuring 39 x 20 x 25 cm and containing a total volume of 15 liters (L). Diluter delivery was 0.5 L to each aquarium at an approximate rate of 171 times per day for the equivalent of 5.7 aquarium volume replacements per 24-hour period.

Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 16-mesh Nitex® screen bottoms. Incubation cups were suspended in test aquaria (2 cups/aquarium) and were gently oscillated by a rocker arm apparatus in the test water. Aquaria were held in a water bath to maintain test solution temperature at 12 ± 1°C. A photoperiod of 12 hours light and 12 hours dark was maintained using fluorescent lighting with a light intensity of 20-100 footcandles at the water surface.

Characteristics of the dilution water used in this study were: total hardness, 32 to 40 mg/L as $CaCO_3$; total alkalinity, 28 to 36 mg/L as $CaCO_3$; pH, 8.6 to 8.8; and specific conductance, 80 to 130 μmhos/cm.

C. Dosage: This was an 82-day early life stage test.

D. Design: Based on the results of a 14-day preliminary

toxicity test with juvenile rainbow trout, this definitive early life stage test was conducted with nominal test concentrations of 0.031, 0.062, 0.12, 0.25 and 0.50 mg MON 097/L of dilution water. Triethylene glycol (TEG) was used as a solvent in this test. A TEG solvent control was maintained concurrently with MON 097 test concentrations. The solvent concentration was 35 uL/L and equal to the solvent concentration in the highest test concentration. A dilution water control was also maintained.

Following water hardening, 50 embryos were impartially distributed to each of 28 embryo cups, two of which were suspended in each of the duplicate test aquaria representing the five MON 097 exposure concentrations, the solvent (TEG) control and the dilution water control. Embryos were observed during the following 21 days to determine viability and hatchability.

On day 21, the surviving larvae were transferred from the embryo cups into their respective exposure aquarium and the 61-day larval exposure was initiated. Larvae were fed live brine shrimp (Artemia salina) nauplii three times a day on week days and two times a day on weekends and holidays when they began swimming up and actively feeding. On day 42 post-hatch, frozen brine shrimp were substituted for the live shrimp at one of the daily feedings. Behavior and appearance of larvae were observed daily and larvae counted weekly. At 61 days post-hatch, all larvae were anesthetized and percent survival, total length and wet weight were determined.

Dissolved oxygen concentration, pH, and temperature were measured in each aquarium on day 0. Thereafter, these parameters were measured daily in both replicate aquaria of one test treatment with the treatment alternated so that each aquarium was measured once each week.

Both replicates of the control, high, middle and low test concentrations were sampled two days prior to test initiation. All MON 097 test solutions and both controls were sampled on test day 0 and weekly thereafter for the duration of the test. Two QA blind samples were also prepared at each sampling interval and were carried through the extraction and analysis procedures used with all the other samples. MON 097 in samples was measured using a gas chromatograph equipped

with an electron-capture detector.

E. Statistics: Percent embryo viability and hatching, and larval survival and growth (length and weight) data were subjected to an analysis of variance (ANOVA). Data for percent hatching and percent survival were arcsine square-root transformed before the ANOVA. If effects due to treatments were indicated, then the means for these endpoints from MON 097 treatments were compared to those from the control and solvent control using Dunnett's procedure. If a treatment effect was significantly different (P = 0.05) from the control, then that treatment was considered to be an effect level. The MATC for MON 097 to rainbow trout embryos and larvae was estimated from this analysis.

12. REPORTED RESULTS: Mean measured MON 097 concentrations approximated nominal concentrations closely, averaging from 90 to 106 percent (Table 5, attached). Variability over time at each concentration was not large, with the standard deviation ranging from 8 to 13 percent of the mean value for 28 samples at each MON 097 treatment.

Water quality characteristics measured during this test were generally consistent with dissolved oxygen concentrations ranging from 9.4 to 9.9 mg/L; temperature, 12 $\pm$ 0°C; hardness, 36 mg/L as $CaCO_3$; and pH, 7.2 to 8.7 (Table 4, attached).

Percent embryo viability and hatchability were not affected by exposure to MON 097 mean measured concentrations as high as 0.45 mg/L, the highest concentration tested. Similarly, survival and average wet weight of larvae were not affected at any MON 097 concentration tested. Observations of larvae lethargy for the first 14 days of exposure did not continue thereafter. Mean total length of trout larvae at 0.033 mg/L MON 097, the lowest concentration tested, was not different from that of dilution water controls but was significantly different from solvent controls (Table 6, attached).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The authors concluded that these apparent effects were not MON 097 related. Based on the lack of statistically significant effects of MON 097 exposure, the MATC for MON 097 to rainbow trout was reported as >0.45 mg/L, the highest concentration tested.

The signature page of this report states that data were audited on December 29, 1982; February 1, March 7, April 25,

May 3 and 4, 1983 by the Director of the Quality Assurance Unit.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the SEP, except for the following deviations:

- The author did not state: 1) whether the eggs and sperm were from at least three females and three males; 2) whether the embryos were at the eyed stage before test initiation.
- The test included only two replicates per concentration, instead of four replicates as recommended by the SEP.
- A photoperiod of 12L/12D was used during the exposure period, instead of 16L/8D as recommended by the SEP.
- It appears that the fish were fed until the end of the test. Fish should not be fed at least 24 hours prior to test termination.
- The mean body weight for larvae in each aquarium was calculated from the total wet weight of all individuals in the aquarium. The fish should have been weighed individually to account for variability among fish within a replicate.
- Raw data were not submitted with the report for statistical validation.

B. Statistical Analysis: The test results could not be validated because the necessary raw data were not provided.

C. Discussion/Results: The study appears to have been performed in a technically sound manner. Since no raw data were provided, the author's statistical analyses could not be verified. Based on the author's conclusions, the MATC for rainbow trout was >0.45 mg/L mean measured concentration, the highest concentration tested. If the author's statistical analyses are valid, this study will not fulfill the requirements for a fish early life-stage test because an MATC could not be determined from the test. According to the SEP, "one concentration selected must adversely affect a

life-stage and one concentration must not affect any life-stage."

D. Adequacy of the Study:

(1) Classification: Invalid

(2) Rationale: An MATC could not be determined from the test results. In addition embryo viability in control groups was too low.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes. June 11, 1990.

ACETOCHLOR

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