(3-16-92)

MRID No. 419764-05

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorsulfuron.
Shaughnessey No. 118601.

2. **TEST MATERIAL:** DPX-W4189-170; Benzenesulfonamide, 2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-; CAS No. 64902-72-3; 97.9% active ingredient; an off-white solid.

3. **STUDY TYPE:** Freshwater Fish Early Life-Stage Test.
Species Tested: Rainbow trout (*Oncorhynchus mykiss*).

4. **CITATION:** Pierson, K.B. 1991. Flow-Through, 77 Day Toxicity of DPX-W4189-170 to Embryo and Larval Rainbow Trout, *Oncorhynchus mykiss*. Report No. 494-91. Study conducted by E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Submitted by E.I. du Pont de Nemours and Company, du Pont Agricultural Products, Wilmington, Delaware. EPA MRID No. 419764-05.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 2/11/92

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 3/12/92

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

Signature: Michael Rexrode
Date: Henry T. Craven 3/10/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a fish early life-stage toxicity test. The MATC of DPX-W4189-170 for *Oncorhynchus mykiss* was >31.1 and <64.8 mg/l time-weighted average concentrations (geometric mean MATC = 44.9 mg/l). 3/16/92

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Rainbow trout (*Oncorhynchus mykiss*) eggs and milt were obtained from Aquatic Research Organisms, Hampton, New Hampshire. Eggs and milt were shipped in separate containers on ice (8.5°C) and, upon receipt in the laboratory, were allowed to warm up to 11.5°C. The eggs were allowed to sit for 15 minutes after fertilization. The embryos were rinsed with dilution water (13°C) to remove debris and excess milt and held in water (11.5°C) until test initiation. At the time of test initiation, the embryos were 20 hours post-fertilization.

B. **Test System:** The test system was an intermittent proportional diluter which delivered selected test solutions to replicate test vessels every 17 minutes. Glass test aquaria (41 x 20.5 x 26 cm) were divided into two replicate chambers, each with a solution depth of 18 cm and a solution volume of 7 l. Embryo incubation cups (5.5-cm diameter) were 212-ml glass screen-bottom cups. Two incubation cups were placed in each replicate chamber using random numbers.

The photoperiod used during the study was 16 hours of light and 8 hours of darkness. The light intensity was 2.15 lux prior to and during the hatching stage and 86-140 lux after swim-up. Thirty-minute transitional light periods were provided. The test temperature (10 ±2°C) was maintained by a water bath.

The dilution water originated from the laboratory well and, at test initiation, had a pH of 7.1, a specific conductivity of 135 µmhos/cm, and a hardness and alkalinity of 75 and 84 mg/l as $CaCO_3$, respectively.

Stock solutions (1,000 mg/l) were prepared daily. NaOH solution (approximately 26-36 ml) was added to 200 l of dilution water to adjust the pH of the dilution water to 9.0. The test material was then added to the pH-adjusted water and stirred for approximately 45 minutes during which time the pH of the stock solution dropped. The pH of the stock solution was adjusted to approximately 7.5 with NaOH. Undiluted stock solution provided the highest test concentration (1000 mg/l)and proportional dilutions of this stock provided the remaining test concentrations.

C. **Dosage:** Seventy-seven-day, embryo-larval, flow-through test. Nominal test concentrations selected based on results of a range-finding study were 15.6, 31.3, 62.5, 125, 250, 500, and 1,000 mg/l. A dilution water control was also included.

D. **Design:** Twenty embryos (20 hours post-fertilization) were added to each of two incubation cups in each replicate chamber (i.e., 80 embryos/concentration). Incubation cups were oscillated at 1.6 cycles per minute. Dead eggs in each cup were recorded and removed daily until day 11. Beginning on day 12, the number of live embryos was determined until hatching was complete on test day 32. Alevins remained in the egg cups until swim-up was completed. Following swim-up, fingerlings were pooled then randomly thinned to 15 per replicate (30 larvae/concentration). At thinning (day 44), standard length of discarded larvae and survival was recorded. At test termination (day 77), survival and growth (standard length and wet weight) were determined. At test termination, the biomass loading in the A replicate of the control was 0.016 g/l/day.

Beginning on test day 45, larvae were fed newly hatched *Artemia* three times daily *ad libitum*. From day 54 on, frozen brine shrimp were substituted *ad libitum* three times daily Beginning on day 61, frozen brine shrimp were provided twice daily. Uneaten food was removed when necessary.

Dissolved oxygen (DO) concentration and pH were measured in each replicate. Total alkalinity, EDTA hardness, and conductivity were measured in the A replicate of the control. These parameters were recorded immediately prior to test initiation and weekly during the study. Temperature in each replicate was monitored weekly using a mercury thermometer and temperature variation in the control was monitored continuously using a recording device.

The diluter system was started approximately 48 hours prior to test initiation and diluter operation was checked daily. During the study, samples were collected on test days -1, 0, 6, 13, 20, 27, 34, 36, 41, 48, 55, 57, 62, 69, and 77 for determination of DPX-W4189-170 concentrations using high performance liquid chromatography.

E. **Statistics:** Parameters undergoing statistical analysis were percentage embryo survival at day 44, larval survival at day 44 and 77, length of larvae at day 44 and 77, and weight of larvae at day 77.

Percentage survival data were evaluated with the Cochran-Armitage procedure. The test was applied to data from all treatments. If a significant difference was determined, the data from the highest concentration were deleted and the analysis was repeated. If no significant difference was determined, the analysis was stopped.

Length and weight data were found to be non-normal based on a Shapiro-Wilk test. Analysis was performed using a Kruskal-Wallis test followed by multiple comparisons. For length at thinning, permutation tests were done for Kruskal-Wallis and multiple Mann-Whitney tests with a Bonferroni's correction for multiple comparisons. All conclusions of statistical significance were based on a $p \leq 0.05$.

12. **REPORTED RESULTS:** The mean measured concentrations of DPX-W4189-170 established in the test solutions were 18, 32, 66, 120, 250, 470, and 900 mg/l (Table I, attached). These values represent 90-115.6% of the nominal exposure concentrations. No sign of undissolved test material was observed in the test solutions or diluter mixing chamber.

Behavior potentially correlated with toxicity of fish was noted at the two highest test concentrations (470 and 900 mg/l). Percentage embryo survival in treatments ranged from 60% to 80%. Survival of embryos and alevins at all concentrations at the conclusion of swim-up (day 44) was not significantly different from that of the controls. At test termination, larval survival in the control and test concentrations ranged from 80% to 100% and was significantly reduced at the highest concentration. Standard length at thinning was significantly reduced at $\geq$470 mg/l mean measured concentration. At test termination, weight was significantly reduced at $\geq$470 mg/l mean measured concentration. Standard length at test termination was significantly reduced at $\geq$66 mg/l.

During the study, the control had a specific conductance of 135-150 μmhos/cm, a temperature of 8.8-11.3°C, and hardness and alkalinity ranges of 73-86 and 80-97 mg/l as $CaCO_3$, respectively. For all treatments, the DO concentrations

ranged from 7.9 to 11.2 mg/l, pH from 6.9 to 7.7, and temperature from 8.3 to 11.6°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Length showed the greatest sensitivity to DPX-W4189-170 exposure. The NOEC for DPX-W4189-170 in a 77 day, flow-through, embryo/larval test using rainbow trout (*Oncorhynchus mykiss*) and mean measured DPX-W4189-170 concentrations was 32 mg/l based on standard length. The LOEC was 66 mg/l, and the MATC was 46 mg/l."

A Good Laboratory Practice Statement, signed by the study director and a company representative, was included in the report indicating that this study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations for FIFRA (40 CFR 160). The report also included Quality Assurance Documentation which was signed by a quality assurance auditor.

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

A. **Test Procedure:** The test procedure is generally in accordance with the SEP and ASTM guidelines, except for the following deviations:

Two measured concentration values in the 32 and 900 mg/l mean measured concentration exceeded 30% of the time-weighted average measured concentration for more than 5% of the test period. However, it is probably acceptable for this study since these fluctuations occurred at test initiation (day 0) and just after first hatch (day 13), respectively. In addition, these concentrations were not a limit value of the MATC.

The measured concentrations of some samples collected on test day 36, 57, and 69 were not included in calculation of the mean measured concentration. All measured concentrations should be included. However, the time-weighted averages of measured concentrations calculated by the reviewer were 16.8, 32.1, 64.8, 110.9, 249.4, 443.5, and 905.4 mg/l and were similar to the means presented by the authors. The reviewer's means will be used for reporting the MATC of this test material.

On page 146 of the report, the test material is described as an off-white solid. On page 157, a footnote of the data sheet describes the test material as a fine powder. This is a discrepancy in the report.

Maximum feeding frequency was 3 times daily *ad libitum*; ASTM guidelines recommend 4 times daily.

Fish weight was measured as wet weight; dry weight is preferred.

The author did not report the number of males and females involved in producing the embryos used in this study; the SEP recommends at least 3 males and 3 females.

The report does not indicate whether the dilution water was intensely aerated prior to addition of the test material. However, the DO range in the test solutions during the study is acceptable.

The conductivity, alkalinity, and hardness of at least one test concentration were not reported. The SEP recommends these parameters be measured for the control and one test concentration.

The light intensity employed in this study was approximately 86-140 lux. The SEP recommends an intensity of 400-800 lux.

B. **Statistical Analysis:** The embryo and larval survival data (arcsine square-root transformed) of each treatment level were compared with those of the control using a one-way analysis of variance (ANOVA) with Dunnett's test. Analyses of these data demonstrated no significant difference between the control and any test concentration (printout, attached).

Growth data were analyzed using a two-way ANOVA and Dunnett's test. Length at thinning was reduced at the highest test level (905.4 mg/l time-weighted average concentration). Wet weight at test termination was reduced at the 2 highest levels (443.5 and 905.4 mg/l time-weighted average concentrations). Length at test termination was reduced at the 5 highest test levels (64.8-905.4 mg/l time-weighted average concentrations). These results are similar to those of the author.

C. **Discussion/Results:** The deviations cited above probably did not affect the results of the study. The author used a one-way ANOVA to analyze the length and weight data. When a one-way ANOVA is used, the variation that exists within replicate vessels is ignored. A two-way ANOVA should have been used.

This study is scientifically sound and meets the guideline requirements for a fish early life-stage test. The MATC of DPX-W4189-170 for rainbow trout was >31.1 and <64.8 mg/l time-weighted average concentrations (geometric mean MATC = 44.9 mg/l).

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, February 5, 1992.