(7-20-94)

ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT

1. **Chemical:** Cimectacarb - Shaughnessy Code 112602

2. **Test Material:** Trinexapac ethyl, 93.8% technical

3. **Study Type:** 21 day chronic toxicity study using Daphnia magna under flowthrough conditions **FIFRA Guideline 72-4**

4. **Study Identification:**
   Study Director: Putt, Arthur E.
   Study Laboratory: Springborn Laboratories, Wareham, Mass
   Study Dates: May 3 - 24, 1993
   Study Identification: Study ID:. 1781.0393.6361.130
   Study Sponsor: Ciba-Geigy Corporation
   EPA Identification: MRID 43128602

5. **Reviewed by:** Brian Montague, Fisheries Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division (H507C)
   Brian Montague 7/20/94

6. **Approved by:** Les Touart, PhD, Section Supervisor
   Ecological Effects Branch
   Environmental Fate and Effects Division

7. **Conclusions:** This study is acceptable for registration guideline satisfaction of Freshwater Invertebrate Lifecycle testing requirements. Significant reductions in growth(length) were seen at 5.1 mg ai/L of Cimectacarb. The LOEC is $\leq$5.1 mg ai/L and the NOEC is $\geq$2.4 mg ai/L based on the results of this study.

8. **Recommendations:** N/A

9. **Submission Purpose:** This study was submitted to satisfy registration ecotoxicity testing requirements for Cimectacarb- a growth regulator for use on turf.

10. **Study Design and Protocol:** The protocol follows EPA/FIFRA Guidelines as specified in the Standard Evaluation Procedure issued by the Hazard Evaluation Division of EPA's Office of Pesticide Programs.

Dilution Water and Test Solutions Preparations: Culture and test solution water were prepared in 1900-liter batches by fortifying well water according to a formula for hard water and filtering it through resin column and carbon filtration system to remove any potential organic contaminants. Fortified water was discarded if not used with 14 days of preparation. Culture water generally had a total hardness and alkalinity as calcium carbonate of 160 to 180 mg/L and 110 to 130 mg/L, respectively, a pH range of 7.9 to 8.3, and a specific conductivity of 400 to 600 micromhos per centimeter. The TOC concentration of the dilution water source was 1.4 mg/L for May, 1993. Several species of daphnids have been cultured in water from the same source as the dilution water utilized in this study.

The test material was heated to 36°C for approximately 15 minutes to liquify it prior to test initiation. The test material sample was liquified only once and fifteen individual aliquots (7.996 g per aliquot) were weighed after liquification for use in the stock preparations for the definitive exposure. A 50 mg A.I./L diluter stock solution was prepared every 48 hours by diluting 7.996 g of CGA-163935 Technical with 150-L dilution water in a 200-L Nalgene® vat. The resultant stock solution was observed to be clear and colorless. The stock solution was then stirred for 46 $\pm$ 2 hours. The 50 mg A.I/L stock solution was transferred to a 165-L glass aquarium for use in the diluter system.

Test Organisms: The *Daphnia magna* used during this toxicity test were obtained from laboratory cultures maintained at Springborn Laboratories, Inc. The daphnid culture area received a regulated 16D/8N photoperiod at an intensity of 34 to 44 footcandles at the surface. Culture vessels were maintained in a temperature controlled water bath designed to maintain solution temperatures at 20$\pm$2°C. Daphnids were fed a combination of a trout food suspension and a unicellular green alga (*Ankistrodesmus falcatus)* once daily. The daphnid were also fed a dietary supplement, Selco® (a commercial mixture of proteins and fatty acids, 0.60 mg/mL) during the exposure period.

Test Material and Design: A Mount and Brungs type intermittent-flow proportional diluter system delivered the dilution water and the test material to the exposure vessels. The diluter was constructed entirely of glass with silicone tubing, stoppers and sealant. A pre-dilution chamber was calibrated to deliver 390 mL/cycle of the Cimectacarb technical stock solution (50 mg A.I./mL) to the diluter mixing chamber which was stirred continuously with a magnetic stirrer. The solution was proportionally diluted (50% dilution factor) to provide the remaining nominal test concentrations,: 3.1, 6.3, 13 and 25 mg A.I./L. Diluter function was visually checked twice daily during the definitive study.

Test vessels were 1.6 liter glass battery jars. Exposure solutions drained from each vessel through two 2-cm holes approximately 15 cm from the bottom of the jars which maintained the test solution volume at 1.4 liters. Nitex® 40-mesh screen covered drainage openings to prevent loss of test daphnids. A dilution water control was included. All treatments and the control were maintained in quadruplicate. Test solutions delivery was at an approximate rate of 6 test chamber volumes per 24-hour period (90% test solution replacement/9 hours). The study was conducted in a water bath designed to maintain the test solution temperatures at 20±1°C.

At initiation test daphnids ≤ 24 hrs old were impartially selected and distributed to 28 unlabeled intermediate vessels containing 40 mL of dilution water and several drops of algal food solution. Daphnid were added two at a time to intermediate vessels until each contained ten organisms. The daphnids were then introduced into the replicate exposure vessels by impartially selecting of the unlabeled intermediate vessels containing ten organisms and gently pipetting them one at a time under the surface of the test solution. Each test concentration then contained forty *Daphnia magna* (10 per replicate).

Survival of adult daphnids was determined on test days, 1,2,4,7,9,11,14,16, 18 and 21. Offspring counts were made on day 7 and three times per week thereafter until test termination. After each observation interval, the offspring removed were counted and discarded. At test termination, the length and weight of each surviving adult daphnid was measured. Following the length measurements, daphnids were dried in an oven at 60°C for 72 hours and then individually weighed (to the nearest 0.01 mg).

During the exposure, daphnids were fed three times daily on weekdays and twice daily on weekends. Exposure vessels were cleaned a minimum of twice each week. Temperature was

measured daily in one replicate of each treatment level and control throughout the 21-day exposure. The temperature was continuously monitored in one replicate of the 50 mg A.I./L. The dissolved oxygen concentrations were measured every weekday in one replicate vessel from each treatment level. Total hardness, alkalinity, pH and specific conductivity of the test solutions were monitored at test initial and weekly thereafter in one replicate vessel from each treatment level and the control solutions.

The dilution control and the high, middle and low test concentrations were sampled and analyzed for Cimectacarb concentration twice prior to the initiation of the definitive test and on test days 0, 7, 14, and 21 during the definitive phase of the test. Samples were removed from alternate replicate test vessels at each sampling interval. Three Quality Control samples were also prepared at each sampling interval.

11. Reported Test Results

Preliminary Testing: *Daphnia magna* continuously exposed for 21 days to mean measured concentrations of 2.9, 6.0, 11, 21 and 42 mg A.I./L showed survival levels which ranged from 88 to 98%. These values were not statistically different from the survival of the control organisms (98%). Reproduction among daphnids exposed to the 6.0, 11 and 21 mg A.I./L exposure solutions averaged 168, 165 and 151 offspring per female, respectively (not statistically reduced in comparison to control daphnids). Reproduction averaging 112 offspring per female was observed in the 2.9 mg A.I./L treatment level which was determined to be significantly reduced in comparison to control organisms. However, similar reductions in the next three highest concentrations were not observed.

Dry weight among daphnids exposed to the 21 and 42 mg A.I./L treatment levels averaged 1.2 and 1.1 mg, respectively. Statistical analysis established a significant reduction when compared to the mean dry weight of control organisms (1.6 mg). A statistically significant reduction was also established in both length (5.3 mm) and dry weight (0.54 mg) of daphnids exposed to the lowest concentration tested (2.9 mg A.I./L). In an effort to verify the No-Observed-Effect Concentration obtained during this exposure, a definitive exposure was conducted at nominal concentrations of 3.1, 6.3, 13, 25 and 50 mg A.I./L.

Definitive Test. Analysis of the exposure solutions for the test material during the pretest period resulted in measured concentrations which averaged 82% of nominal. No undissolved test material was observed in the diluter system or exposure solutions during the chronic study. The analysis of exposure

solutions for Cimectacarb during the in-life portion of the definitive test were generally consistent between replicate vessels. Based on mean measured concentrations (which averaged 81% of nominal) the treatment levels were defined as 2.4, 5.1, 10, 21 and 43 mg A.I./L.

Quality Control samples established recoveries which averaged 100% of the nominal fortified levels (3.00 to 50.0 mg A.I./L, 12 samples). At termination, survival of 95, 68, 58, 83 and 95% was observed among daphnids exposed to the 2.4, 5.1, 10, 21 and 43 mg A.I./L treatment levels respectively. The 21-day EC_{50} was empirically estimated to be >43 mg A.I./L, the highest concentration tested, due to the highest survival at 21 and 43 mg ai/L concentrations. Daphnids throughout the treatment range released their first brood on day 8, which was consistent with control performance. Daphnids exposed to the 10, 21 and 43 mg A.I./L test levels released an average of 161, 175 and 144 offspring per female, respectively. Reproduction in the 2.4 and 5.1 mg A.I./L treatment levels averaged 224 and 187 offspring per female. No young were observed to be immobilized in any concentration tested or in the control group.

Mean total body length at test termination among daphnids exposed to the 10, 21, and 43 mg A.I./L test levels averaged 5.2, 5.2 and 5.4 mm, respectively. This was shown to be statistically different from the mean total length of control daphnids (5.5 mm). Mean total length of daphnids exposed to the 5.1 mg A.I./L concentration averaged 5.3 mm. Daphnids in the 2.4 mg A.I./L test group showed an average body length of 5.5 mm. Mean dry weight of daphnids following 21 days exposure to the 43 mg A.I./L and 21 mg A.I./L test concentrations averaged 1.57 mg and 1.44 mg, respectively. This was comparable to the dry weight of the control organisms (1.48 mg). The dry weight of daphnids exposed to exposure levels of 5.1 and 10 mg A.I./L were less than expected (1.28 and 1.18 mg, respectively). Daphnids exposed to the highest treatment level weighed more than the control organisms. Daphnids exposed to the lowest treatment had an average dry body weight of 1.36 mg.

12. **Study Author's Conclusions:** "Based on the data generated during this study, it was determined that daphnid survival was unaffected by exposure to CGA-163935 concentrations ranging from 2.4 to 43 mg A.I./L. Daphnid reproduction was adversely affected by exposure only to concentrations of 10, 21 and 43 mg A.I./L. Reproduction among daphnids exposed to concentrations of 2.4 and 5.1 mg A.,I./L was unaffected relative to the control organisms performance. Organisms growth, determined as total body length, was the most sensitive parameter measured. Organisms exposed to

concentrations of 5.1 mg A.I./L and above had reduced lengths compared to controls. Since organisms exposed to the highest exposure level (43 mg A.I./L) had body lengths and weights which were comparable to the body size of the control organisms, variability observed at lower treatments may not be in response to the toxicity of the test article. Although, it is probable that the No-Observed-Effect Concentration for this study is 5.1 mg A.I./L, the highest concentration which did not affect organism reproduction, a conservative estimate of the NOEC for CGA-163935 was estimated as 2.4 mg A.I./L, based on length. The MATC for this study was determined to be >24 mg A.I./L and <5.1 mg A.I./L (Geometric Mean MATC = 3.5 mg A.I./L)."

13. **Reviewer's Discussion:** Agency analysis of 21 day adult survival, 21 Day offspring/female, 21 day length and 21 day dry weight produced the following results.

No significant adult mortality attributable to the test material, though there was a significant reduced survival level in the 10 mg/L test group.

Significant reduction in offspring produced per female was seen in the 43 mg/L test level. Slight but not statistically significant reduction was seen in offspring per female produced in the 21 mg/L test concentration.

Significant reductions were seen in the 21 day length of adult daphnids at concentrations over 2.4 mg ai/L..

Though significant reductions were seen in mean weight of daphnids in 2.4, 5.1, and 10 mg/L dose levels these were generally due to the effects of replicates which should be considered outliers. The 21 and 43 mg/L concentrations showed comparable mean weight values with the control daphnids. Therefore, the reductions are not felt to follow a dose response curve and were probably not dose related.

Based on these conclusions the Agency has determined that the LOEC for Cimectacarb is 5.1 mg/L and the NOEC is 2.4 mg/L.

Adequacy of Study:
Category: Core
Rationale: Study results support the study director's conclusions.
Repairable: N/A.