

(9-23-92)

EEB Data Evaluation Report

1. Chemical: Cimectacarb

2. Test Material: Cimectacarb technical (CGA-163935)  
Active Ingredient: 4-(cyclopropyl-x-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethyl ester;.....92.2%  
Inert Ingredients:.....7.8%

3. Study Type: 21-day Chronic Toxicity Test with Daphnia magna under flow-through conditions.

4. Study Identification:

Study Author: Putt, Arthur E.  
Study Laboratory: Springborn Laboratories, Wareham, Mass.  
Study Dates: January 31 - February 21, 1991  
Sponsor: Ciba Geigy Corporation  
Laboratory Identification: Study No. 91-3-3708  
EPA Identification: MRID 418695-12

5. Reviewed by: Brian Montague, Fisheries Biologist  
Ecological Effects Branch  
Environmental Fate and Effects Division

*Brian Montague* 8/26/92

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

*L. Touart*  
9-23-92

7. Conclusions: The study provides useful data concerning effects on reproductive performance, but has failed to establish an NOEL at which reproductive impairment begins. Reproductive impairment was seen at all treatment levels tested ( $\geq 2.9-42$  mg/L). Though weight loss was not seen in a dose response progression the Branch does not feel that this effect can be completely dismissed at the 2.9, 21, and 42 mg ai/L concentrations.

8. Recommendations: Testing should be repeated at lower concentrations to completely satisfy this guideline requirement.

concentrations.

9. **Study Purpose:** Study was submitted in support of registration for Cimectacarb, a new growth regulator.
10. **Study Design and Protocol:** Protocol was based on FIFRA guideline 72-4 protocol requirements as explained in SLI Protocol #010190. Two amendments were added to the original protocol.

**Test Organisms:** Laboratory cultured daphnids were obtained from laboratory cultures. The daphnids were raised under static renewal conditions at 20±2°C in fortified well water. Green algae culture and trout food suspension were fed to daphnids until use for definitive testing. First instar daphnids <24 hours old were chosen for use in this study.

**Test Dilution Water and Solutions:** Test water was prepared in 1900 liter batches as per ASTM guidelines for preparation of hardwater from wellwater.

Diluter stock solutions of 50 mg AI/L were prepared every other day by dilution of 7.496 gms ai into 150L of dilution water. After 48 hours of stirring solutions were used. Test material appeared to remain in solution. A Mount and Brungs designed intermittent-flow proportional diluter delivered dilutions of the highest nominal treatment level, 50 ppm, in 50% dilutions of 25, 13, 6.3, and 3.1 ppm. Solutions were delivered to 1.6 liter glass test vessels with overflow draws to maintain 1.4L volume. Six volume exchanges were introduced per 24 hours. A 16D/8N photoperiod at 26-90 fc was maintained. Temperature controlled water bath maintained test vessels at 20±1°C.

Offspring reproduction was recorded on days 8, 11, 14, 15, 18 and 21. Measurement of body length and dry weight was made at test termination. During the treatment period 2.0 ml of trout food suspension, 3.0 ml of algae suspension and 0.5 ml of commercial protein/fatty acid supplement were provided three times/day Mon-Fri and twice daily on weekends or holidays. Temperature was measured daily in one replicate/treatment or control with continuous monitoring in one vessel. D.O was measured every weekday in alternate replicates. pH, temperature, and D.O. were obtained for all vessels on a weekly basis. Water samples removed on days, 0, 7, 14, and 21 were later analyzed using HPLC procedures.

11. **Reported Test Results:** Water quality parameters generally remained stable according to reported 0, 7, 14, 21 day measurements. The pH in all vessels ranged from 8.3 to 7.9. D.O. dipped to 6.8 (day 14) down from 8.5 (day 0), but later returned to 7.4 on day 21 in the highest test level. Other treatment levels generally remained above 7.4 mg/L of D.O. Temperature ranged from 19-21°C. Measured concentrations recoveries were approximately 90% of the estimated nominal levels and mean measured concentrations for all measurements were 42, 21, 11, 6.0, and 2.9 mg/L. Apparently the lowest level of detection for the analytical method employed was 1.5 ppm as control measurements are listed as <1.5 mg ai/L. All prepared test solution aliquots were discarded if not used within 14 days. Hardness and alkalinity were 160-180 mg/L and 110-130 mg/L as CaCO<sub>3</sub>, respectively. pH ranged from 7.9 -8.3 and conductivity was 400-600 micro ohms/cm. No pesticides or PCB's were detected in analysis of sample water. TOC during the test period was 1.5 mg/L.

Prior to definitive testing 21 day range finding studies were conducted (results > 90% survival at >150 ppm). For definitive testing 10 daphnids per replicate vessel were impartially selected and added to each of the 24 test vessels. Survival was recorded on days 1,2, 4, 7,8, 11, 14,16,18 and 21..

The mean percent survival of adult daphnids ranged from 88% to 98% thus the 21 day EC50 was estimated to be >42 ppm. Mean average reproduction/female was 183, 130, 151, 165, 168, 112 offspring/female in the control, 42, 21, 11, 6.0, and 2.9 mg ai/L treatment groups, respectively. Weight reductions of 45, 33, and 66% were noted at 42, 21, and 2.9 mg/L dosage levels. The latter value was not felt to be treatment related (2.9 mg/L level). Length measurements showed no statistically valid differences from the controls.

12. **Study Author's Conclusions:** After 21 days exposure, the control daphnids survived and reproduced at rates which met the minimum standard criteria established by the EPA (1985) under FIFRA Guideline #74-2 (ie  $\geq 70\%$  survival,  $\geq 40$  offspring per female).

In summary, it was established that the adverse effect on organism growth (measured by body wt.) was the most sensitive indicator of toxicity of CGA-163935 to Daphnia magna. Based on this data, The Maximum Acceptable Toxicant Concentration (MATC) of CGA-163935 Technical to Daphnia magna was established to be >11 and <21 mg AI/L (geometric mean = 15 mg AI/L).

13. **Reviewer's Discussion:** Protocol followed acceptable procedural guidelines. Water quality parameters, sampling

Some problems were apparent in the lowest tested dosage as per descriptions of adult behavior, though the next dosage level did not appear to demonstrate any serious behavioral abnormalities. No clear explanation for stress is apparent from water quality records which are provided, but the problems are later reflected in poor reproductive performance in this group.

The results show a significant response in the mean dry weight parameter of adult growth at 21, 42 and 2.9 ppm. Though the study author felt this to be unrelated to the chemical the response was seen in 3 of the 5 test concentrations. Though environmental parameters may have been responsible the Branch does not feel that this has been firmly established. Adult length was not affected significantly. Only the highest concentration daphnids demonstrated erratic swimming behavior.

Reproductive response in the control daphnids was acceptable and reproduction in all treatments was first observed on day 8. Due to low numbers at this point no significant difference could be rated. On days 11, 14, 15, 18 and 21 significant differences in numbers of offspring produced per female in controls vs all treatments is apparent though not always in a clear dose response curve. Significant differences in the percent survival of the percent that were produced is not apparent until day 21 observations where percent survival drops to 88 and 90% in the 42 and 21 ppm test concentrations, respectively. The study author's conclusion that cumulative offspring/female numbers are significantly less than control in the highest dose only are not supported in statistical analysis performed by the Agency for day 11, 14, 15, 18 and 21 observed numbers of offspring/female. If the lowest dose (2.9 ppm) is removed from consideration significant reduction is still seen in all other treatment levels. Thus, a clear NOEL and MATC for offspring produced cannot be established. The study results have shown the compound to reduce numbers of offspring at levels of 6.0 ppm and possibly at 2.9 ppm. Growth parameters for adults are affected at concentrations  $\geq 21$  ppm and possibly at  $\geq 2.9$  mg ai/L.

#### **Adequacy of Study:**

**Classification:** Supplemental

**Rationale:** Though there is useful data provided no indication of an NOEL (and thus a true MATC) has been established as reproduction was affected at all treatment levels.

**Repairability:** Not repairable. Study should be repeated at lower concentration ranges.