12-6-89

RECORD NO.

~~128301~~ 125 301
SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 2/14/89 OUT 12/6/89

FILE OR REG. NO 128301

PETITION OR EXP. NO.

DATE OF SUBMISSION 8/10/88

DATE RECEIVED BY EFED 2/9/89

RD REQUESTED COMPLETION DATE 2/21/89

EEB ESTIMATED COMPLETION DATE 2/21/89

RD ACTION CODE/TYPE OF REVIEW 331

TYPE PRODUCT(S) : I, D, H, F, N, R, S Insect growth regulator

DATA ACCESSION NO(S).

PRODUCT MANAGER NO. P.Hutton (PM 17)

PRODUCT NAME(S) PICTYL

COMPANY NAME Maag Agrochemicals R&D

SUBMISSION PURPOSE Fish early stage and aquatic invertebrate studies submissions; data requirements evaluation

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.
	fenoxycarb	

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

MEMORANDUM

SUBJECT; Fenoxycarb Aquatic Non-Food Use (Mosquito Larvicide)

FROM; James W. Akerman, Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Branch (H7507C)

TO: Phil Hutton, PM 17
Insecticide Rodenticide Branch
Registration Division (H7505C)

Maag Agrochemicals has submitted two studies on chronic aquatic organisms toxicity to support the registration of the use of fenoxycarb as a mosquito larvicide. EEB's evaluation records are attached.

Study	Results	Status
Aquatic Invertebrate Life Cycle (72-4)	MATC = >1.6 ng/L <2.3 ng/L	Core
Fish Early Life Stage (72-4)	MATC = <0.62 mg/L	Supplemental

The above studies were the subjects of previous discussions by EEB (D. Johnson 9/20/88 and 8/26/88) in response to the registrant's intention to develop data to support the fenoxycarb mosquito larvacide use. Additional studies were also prescribed but have yet to be submitted. The following data requirements continue to remain necessary for hazard assessment purposes:

Fish Full Life Cycle with technical test material
Mysid Life Cycle with technical test material
Avian Reproduction with technical test material
Estuarine Fish 96-hour LC_{50} with Pictyl 1E EC
Estuarine Shrimp 96-hour LC_{50} with Pictyl 1E EC
Oyster Embryo-Larvae 96-hour EC_{50} with Pictyl 1E EC

CONCURRENCES								
SYMBOL	J. Noles	H. Craven	H7507C					
SURNAME	H7507C	H7507C						
DATE	11/29/89	11/29/89	11/30/89					

EPA Form 1320-1 (12-70) OFFICIAL FILE COPY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

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The registrant has submitted a fish early life stage study which demonstrated the effects of low fenoxycarb concentrations. However, it was emphasized in the registrant's meeting dated May 10, 1988 and repeated in the 8/26/88 EEB review that the fish full-life cycle study would better address the effects of fenoxycarb on reproduction and larval growth than the fish early life stage study. Rather than to repeat the supplemental fish early life stage study, the registrant should address the fish full life cycle study. The preferred test species shall be an estuarine species, the sheepshead minnow. The use of estuarine species will enable EEB to jointly evaluate the hazards of fenoxycarb to both estuarine and freshwater fishes in their early life stage development. Mosquito larvicides are routinely used in both freshwater and estuarine environments.

The fish early life stage study does indicate that low concentrations of fenoxycarb are capable of impacting early life stage development of fish. As a result, the fish full life cycle must be required to further assess effects of fenoxycarb on reproduction. The registrant and the conducting laboratory is advised to closely examine solvent selection and toxicity because the solvent used in the fish early life stage study appeared to have a toxic effect. As a result, EEB used the lowest value derived from the comparison of the nonsolvent control with the treatment group. This comparison resulted in an MATC (< 0.062 mg/l) below the lowest concentration tested. EEB will have to rely on this value for hazard assessment purposes until the results of the fish full life cycle determine otherwise. The registrant may repeat the fish early life stage study to accurately determine the MATC, in addition to conducting the fish full life cycle.

EEB's files and personal communication with the PM staff indicates that the registrant has not submitted a formal request for the mosquito larvicide use registration. The registrant is pursuing the development of the necessary database before submitting a formal registration request. Once the formal request and required studies are submitted and reviewed, EEB's hazard assessment can be conducted and the need for field studies can be evaluated and designed.

John Noles, Biologist
Ecological Effects Branch

MRID No. 40361806

DATA EVALUATION RECORD

1. **CHEMICAL:** Fenoxycarb
   Shaughnessey Number: 125301

2. **TEST MATERIAL:** Fenoxycarb Technical; Batch No. 13-10-10-32; 93% active ingredient; a tan powder

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

4. **CITATION:** McAllister, W.A. 1987. Early Life Stage Toxicity of Fenoxycarb to Rainbow Trout (Salmo gairdneri) in a Flow-Through System. ABC Report No. 35215. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. Submitted by Maag Agrochemicals, Vero Beach, Florida. MRID No. 40361806

5. **REVIEWED BY:**

   Kimberly D. Rhodes
   Associate Scientist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: Kimberly D. Rhodes
   Date: May 8, 1989

6. **APPROVED BY:**

   Prapimpan Kosalwat, Ph.D.
   Staff Toxicologist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: P. Kosalwat
   Date: May 8, 1989

   Henry T. Craven
   Supervisor, EEB/HED
   USEPA

   Signature: John Noles
   Date: 11/7/89 Henry Craven 11/29/89

7. **CONCLUSIONS:** This study appears to be scientifically sound as Supplemental data and does not fulfill the guideline requirements for a fish early life stage toxicity test. The MATC of Fenoxycarb Technical for rainbow trout (Salmo gairdneri) was < 0.062 mg/l, the lowest concentration tested and measured.

8. **RECOMMENDATIONS:** N/A

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9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Rainbow trout eggs (Salmo gairdneri) were obtained from a commercial supplier in California.

B. Test System: A proportional diluter system described by Mount and Brungs, utilizing a Hamilton Micro Lab 420 syringe dispenser, was used for the intermittent introduction of a dimethylformamide (DMF) solution of Fenoxycarb Technical to four replicate test chambers per concentration. The proportional diluter system used for the project was set to provide test levels approximately 50 percent dilutions of each other. The diluter delivered an average rate of approximately 63 mL/minute/replicate of test solution or control water to the test vessels which was sufficient to replace a replicate volume 9.4 times in a 24-hour period. Five concentrations of the test material with a dilution water control and solvent control were tested. The test chambers were immersed in a temperature controlled water bath held at 12 ± 2°C. During the testing period, the rainbow trout fry were on a 16-hour daylight photoperiod with the eggs shielded from excess U.V. light exposure. The light intensity was 158 ± 25 foot-candles at the water surface.

Each glass aquarium measured 25 x 16 cm with a water depth of 24 cm, yielding an approximate 9.6-liter replicate-chamber volume. The rainbow trout eggs were incubated in cups suspended in the treatment and control water. These egg incubation cups were made from 7-cm diameter tubing with stainless steel screening (16 mesh) fused to the bottom. To insure exchange of water, the egg cups were oscillated in the test solution by means of a rocker-arm apparatus driven by a 4-rpm electric motor.

Dilution water for the rainbow trout test was aerated well water characterized as having a pH of 7.8 - 8.3, total hardness of 225 - 275 mg/L as $CaCO_3$, total alkalinity of 325 - 375 mg/L $CaCO_3$ and specific conductance of 700 umhos/cm.

C. Dosage: 74-day flow-through early life stage test.

D. **Design:** A control, solvent control, and five nominal Fenoxycarb Technical concentrations of 0.06, 0.12, 0.25, 0.50, and 1.0 mg/L based on active ingredient were tested. The solvent control solution contained the maximum amount of DMF present in any test concentration (0.014 mL/L). Thirty rainbow trout eggs were randomly introduced into each replicate chamber (60 eggs per concentration). When hatching commenced, the number of eggs hatched in each incubation cup was recorded daily until hatching was completed. The 60-day post-hatch growth period began when hatching was greater than 95 percent (day 14). On study-day 27 (13 days post-hatch) the rainbow trout sac fry were removed from the duplicate egg incubation cups and separated into 4 replicate growth chambers per concentration. Survival and fry growth data were collected on days 35 and 60 post-hatch. Feeding began on day 27 (13 days post-hatch). The fry were fed brine shrimp nauplii three times each day. Commercial fish food was added to the diet as the fish became larger.

Growth, as determined by standard length of the fry, was determined by the photographic method of McKim and Benoit (1975) on study day 49. At test termination, study day 74 (60 days post-hatch), all surviving fish were measured for standard length and wet weight.

Water quality parameters of dissolved oxygen, pH and conductivity were measured initially on Day 0, Day 1, Day 7 and on every 7th day thereafter until test termination in the control, low concentration, and high concentration. Water hardness and alkalinity were measured on Day 25, Day 49, and Day 74 in the control, low concentration, and high concentration. Temperature was monitored daily and was also continuously recorded with a temperature data logger. The measured concentrations of Fenoxycarb Technical in test water were determined on days 0, 1, 7 and on every 7th day thereafter until study termination on day 74.

E. **Statistics:** Comparison analyses between the control, solvent control, and five test levels were carried out using the measured parameters of hatchability, survival, standard length and wet weight. The statistical data were analyzed by a Systat computer program. Growth data, using the individual per replicate, were analyzed using two-way analysis of variance with an interaction model to determine whether there was any significant effect due to block, i.e.,

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replication. If the analysis indicated no significant interaction, replicate data were pooled for further analysis.

One-way analysis of variance (ANOVA) calculations were used to determine if significant differences existed between the control and treatment levels. If there were no significant differences between the control and solvent control, all individual replicate data were composited by concentration and analyzed by one-way analysis of variance using data from the control. If treatment effects were indicated by a significant F-test of the mean square ratios, Tukey's HSD multiple means comparison test was used to determine which exposure levels differed from the control values.

Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. All differences were considered significant at the 95% confidence level.

12. REPORTED RESULTS: The mean measured concentrations of Fenoxycarb Technical were 0.062, 0.097, 0.20, 0.41, and 0.84 mg a.i./L. The mean measured concentrations ranged from 80 to 103% of the nominal test concentrations.

Hatchability of eyed rainbow trout eggs after 15 days of continuous exposure to Fenoxycarb Technical was significantly lower ($P < 0.05$) in the highest mean measured concentration (0.84 mg a.i./L) when compared to hatch of the control eggs. Hatch was 70% in this level. Mean percentage hatch in the control and solvent control aquaria was 97 and 98% respectively. The percentages in the exposure aquaria were 100% in the first four test levels (Table 8, attached).

The survival of trout continuously exposed to Fenoxycarb Technical after 49 and 74 days is shown in Table 8 (attached). By day 35 post-hatch all fish had died in the highest test concentration and only 1 fish (1.7%) was still alive in the next lower concentration. Survival was not significantly affected in the lowest three test concentrations when compared to the control (88%) and ranged from 90 - 100%. All fish were dead in the two highest test concentrations by 60 days post-hatch but survival was still not affected in the lowest three concentrations and ranged from 90 to 97%. Although the percent survival of fry in the lower Fenoxycarb Technical treated groups was slightly higher than that of the control, it was not statistically significant.

The embryos in the high concentration (0.84 mg a.i./L) that hatched all died shortly after hatch, some appearing to die as soon as the head would emerge. After hatching, the fry in the 0.41-mg a.i./L concentration were extremely quiescent and retained the yolk sac much longer than the fish in the controls and other test levels.

Morphological and behavior abnormalities such as curved spine, fish resting on the bottom of the test chamber, loss of equilibrium, light discoloration, quiescence, enlarged yolk sac and fish swimming vertically in the test chamber were scattered throughout the controls and the three lowest test levels in a small percentage of the fish. By day 50 (36 days post-hatch) most of these effects had either disappeared or the affected fish had died. Quiescence and fish resting on the bottom of the test chamber persisted in a few fish in the 0.20-mg a.i./L concentration until just before test termination.

Results for the effect of Fenoxycarb Technical on length of rainbow trout are shown in Table 8 (attached). The single surviving fish in the 0.41-mg a.i./L concentration was not included in the 35-day post-hatch length analysis since a group that contains only one fish cannot be compared with other groups each containing several fish in an analysis of variance. The 0.20-mg a.i./L concentration fish were the only test fish that showed a significant reduction in length when compared to the control at 35-days post-hatch. By 60 days post-hatch the fish in the lowest three test concentrations all showed a significant ($P < 0.05$) length reduction when compared to the control.

Results for the effect of Fenoxycarb Technical on wet weight of rainbow trout are shown in Table 8 (attached). Growth of the trout fry, as measured by wet weight after 60 days of exposure to Fenoxycarb Technical, was significantly reduced in the lowest three test concentrations.

Water quality parameters of dissolved oxygen, pH and conductivity were considered adequate for testing. On study days 2, 44 and 45, the test temperature as recorded daily on the daily observations forms was 9°C.

Based on the data from this 60-day post-hatch rainbow trout (Salmo gairdneri) early life stage study, the Maximum Acceptable Toxicant Concentration (MATC) of Fenoxycarb Technical was estimated to be < 0.062 mg a.i./L, the lowest concentration tested.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The Maximum Acceptable Toxicant Concentration (MATC) of Fenoxycarb Technical was estimated to be < 0.062 mg a.i./L, the lowest concentration tested.

A GLP compliance statement was included in the report and the study was audited by a QA unit. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards: Pesticide Programs (40 CFR 160).

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o The SEP recommends that test water should have a hardness of 40 to 48 mg/L as $CaCO_3$ and a pH range of 7.2 to 7.6. The hardness of the test water in this study was 225 to 275 mg/L $CaCO_3$ and the pH ranged from 7.8 to 8.3.

o The SEP states that hardness and alkalinity in a control and one concentration must be analyzed once a week. This study only reported these water quality parameters on day 25, day 49, and day 74 of the study period.

o The SEP states that the test temperature should not deviate by more than $2^{o}C$. During this study, the test temperature recorded on day 2, day 44 and day 45, as recorded daily on the daily observations forms, was $9^{o}C$. This is $1^{o}C$ below the temperature test range of $12 \pm 2^{o}C$.

o At 35 days post-hatch, the fry survival in the 0.41-mg/L group was reported as 1.7% in the result section and 6.7% in Table 8 (attached). The reviewer assumes it was actually 1.7% since only 1 fish out of 60 survived at that time.

o The report did not give information on the holding and acclimation conditions of the rainbow trout eggs prior to test initiation.

B. **Statistical Analysis:** The reviewer evaluated embryo hatchability and larval survival following an arc sine square root transformation of the data. The growth data, standard length and wet weight, were statistically evaluated by ANOVA without any transformations. All printouts are attached.

The reviewer confirmed a significant difference at P = 0.01 of hatchability of rainbow trout embryos in the highest mean measured concentration (0.84 mg a.i./L) when compared to the solvent control.

All fish were dead in the two highest test concentrations at test termination. Therefore, the reviewer only evaluated the three lowest test concentrations for survival and growth data. The reviewer confirmed that survival was not significantly affected in the lowest three test concentrations when compared to the solvent control.

The author found that the length and weight of fish at all three lowest test concentrations were significantly lower than those in the combined control groups. The reviewer analyzed the growth data by comparing the treated fish to the control and solvent control fish, separately. When compared to the control, there was a significant reduction in growth (both length and weight) at all treatment levels. However, when compared to the solvent control, only fish lengths at the test concentration of 0.20 mg a.i./L and fish weights at the test concentrations of 0.097 and 0.20 mg a.i./L were significantly reduced.

Therefore, based on the comparison with the solvent control group, the maximum acceptable toxicant concentration (MATC) of Fenoxycarb Technical for rainbow trout embryos and larvae was estimated to be > 0.062 mg a.i./L and < 0.097 mg a.i./L mean measured concentration.

C. **Discussion/Results:** The study results appear scientifically valid. Based on the comparison between the treatment groups and the non-solvent control group, the maximum acceptable toxicant concentration (MATC) of Fenoxycarb Technical for rainbow trout (Salmo gairdneri) embryos and larvae was estimated to be < 0.062 mg a.i./L

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Fenoxycarb Technical for rainbow trout (Salmo gairdneri) embryos and larvae was estimated to be < 0.062 mg a.i./L

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: MATC not determined.

(3) Repairability: Irrepairable. Lowest dosage level showed an effect when compared to the nonsolvent control.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 05-01-89.