DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4

1. CHEMICAL: Benomyl PC Code No.: 099101

2. TEST MATERIALS: Carbendazim (technical) Purity: 99.3%
^{14}C-Carbendazim Radiopurity: 100%

3. CITATION:
Author: J.E. Rhodes, B. Hurshman, and T. Leak
Title: Early Life-Stage Toxicity of Benomyl (as Carbendazim, DPX-E965-299) to the Channel Catfish (*Ictalurus punctatus*) Under Flow-Through Conditions
Study Completion Date: December 1, 1995
Laboratory: ABC Laboratories, Inc., Columbia, MO
Laboratory Report ID: Haskell Laboratory Outside Report 236-95
Sponsor: E.I. du Pont de Nemours and Company, Wilmington, DE
MRID No.: 438728-01
DP Barcode: D221857

4. REVIEWED BY:

Richard C. Petrie, Senior Agronomist
EEB, EFED, OPP

Signature: **Date:**

APPROVED BY:

Ann Stavola, Section Head, Section 5
EEB, EFED, OPP

Signature: **Date:**

6. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for a freshwater fish early life-stage test. Based on the most sensitive parameter (larval survival), the MATC for channel catfish exposed to carbendazim was between 0.99 and 2.11 µg/L (as mean measured, carbendazim concentrations). The geometric mean MATC was 1.44 µg/L.

7. ADEQUACY OF THE STUDY:

A.Classification: Core.

B.Rationale: Although individual growth data were not included in the report, length and wet weight showed no clear dose-response based upon visual examination of the means. Larval survival appeared to be a more sensitive endpoint than growth.

C.Repairability: N/A.

8. MAJOR GUIDELINE DEVIATIONS:

1.Individual growth data were not presented in the report. There must be sufficient information presented in the report for the reviewer to verify the authors' statistical conclusions.

2.The embryo incubation chambers were aerated. Embryo and larval chambers should not be aerated.

3.The dissolved oxygen concentrations during the test were ≥58% saturation. With the exception of Days 35, 38, and 39, most D.O. level were ≥75%. The test system should maintain D.O. concentrations above 75% of saturation.

9. MATERIALS AND METHODS:

A. Biological System

Guideline Criteria	Reported Information
Species: A freshwater or saltwater fish species.	*Ictalurus punctatus*
Source: Commercial fishery, wild, or brood stock.	Newly fertilized embryos were obtained from Osage Catfisheries, Inc., Osage Beach, MO.
Age at beginning of test: Embryos 2 to 24 hours old.	<24 hours post-fertilization at test initiation
Replicates: Minimum of 20 embryos per replicate cup, 4	**Embryo exposure:** 20 eggs/incubation cup,

Guideline Criteria	Reported Information
replicates per concentration. Minimum of 30 fish per treatment for posthatch exposure.	2 cups/replicate aquarium, 4 replicate aquaria/treatment **Larval exposure:** 20 fish per replicate, 4 replicates per treatment
Posthatch: % of embryos that produce live fry must be ≥50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	Percentage hatch was 87.7% in pooled controls (91% and 84.5% in water control 1 and water control 2, respectively).
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Fish were fed at least three times daily. Feeding was terminated 24 hours prior to test termination.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Surviving and dead fry were recorded daily up to Day 21. Thereafter, cumulative mortality was recorded based on observed dead fry. Surviving fry were counted again at test termination.
Controls: Avg. survival at end of test must be ≥80%. Survival in any control chamber must not be <70%.	Average survival of the pooled controls was 89.2%. Survival in each control replicate ranged from 72.2 to 100%.
Controls: Negative control and carrier control (when applicable) are required.	Two sets of dilution water controls were used.

Comments:

B. Physical System

Guideline Criteria	Reported Information
Test Water: 1) May be natural (well or spring) or reconstituted water. 2) Water should be sterilized with UV radiation and screened for contaminants. 3) Hardness of 40-48 mg/L as $CaCO_3$, pH of 7.2-7.6	1) A blend of treated (reverse-osmosis) well water and raw well water. 2) Dilution water was heated, filtered (5-µm), UV-sterilized and screened for contaminants prior to use. 3) Hardness of 132-150 mg/L as $CaCO_3$, pH range of 7.85-8.51.
Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature. For rainbow trout, 10°C is recommended.	Range of 24.2-25.9°C
Photoperiod: Recommend 16L/8D.	Continuous semi-darkness for embryos and 16L/8D after hatching.
Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.	Intermittent-flow proportional diluter. Nominal concentrations: 0.10, 0.22, 0.48, 1.1, 2.3, and 5.2 µg/L as Carbendazim (0.15, 0.33, 0.73, 1.7, 3.5, and 7.9 µg/L as Benomyl equivalent).
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	1) Mixing chambers were used. 2) From test initiation until Day 8, incubation cups were aerated to keep embryos in constant movement and well oxygenated. 3) Appropriate mixing was confirmed by chemical analysis. 4) Flow splitting accuracy was verified prior to test

Guideline Criteria	Reported Information
	initiation and on Day 38. The percentage accuracy was not reported.
Test Vessels: All glass or glass with stainless steel frame.	Glass aquaria (15.5 x 30.5 x 29.2 cm).
Embryo Cups: 120 Ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.	1-liter, narrow-mouth, polyethylene bottles with the bottoms cut out. Two bottles were suspended upside down in each aquarium. The mouth and the hole on the side of the bottle were covered with 15-18 mesh, stainless-steel screen. An airline with an air stone was inserted through the top of the cap to increase circulation.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain D.O. at above 75% of saturation and maintain the toxicant level.	11.9 volume replacements per day until Day 34, then increased to 22.1 volume replacements/day thereafter. D.O. and chemical concentrations were monitored.
Aeration: Dilution water should be aerated to insure D.O. concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.	D.O. $\geq$61% of saturation at all times. Incubation chambers were aerated as discussed above.

Comments: The authors reported an oxygen saturation limit of 7.9 mg/L at 25°C, suggesting a minimum D.O. of 61% of saturation. The reviewer calculated a minimum D.O. of 58% of saturation based on an oxygen saturation limit of 8.25 mg/L at 25°C.

C. Chemical System

Guideline Criteria	Reported Information
Concentrations: Minimum of 5 concentrations and a control,	- Two sets of water controls and six exposure

Guideline Criteria	Reported Information
all replicated, plus solvent control if appropriate. - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage.	concentrations. - Test concentrations were measured in each replicate on Days 0, 6, 14, 21, 28, 35, and 41. - LOEC and NOEC were obtained.
Other Variables: D.O. must be measured at each conc. at least once a week.	D.O. and pH in each replicate were measured weekly until the last 7 days of the study when they were measured daily.
Solvents: Should not exceed 0.1 Ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	No solvent was used.

Comments: None.

10. REPORTED RESULTS:

Guideline Criteria	Reported Information
Data Endpoints must include: - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if appropriate); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs.	Data include: - Number of eggs hatched; - 34-day post-hatch survival; - 34-day post-hatch length; - 34-day post-hatch wet weight.
Raw data included? (Y/N)	Yes, for survival and hatchability data. Only replicate means were reported for growth data.

Effects Data

Mean calculated Benomyl Concentration (µg/L)		Mean Percent Hatch	Survival (34 days Post-Hatch)	Standard Length (mm)	Wet Weight (g)
Nominal	Measured				
Control 1	-	91.0	84.4	43.5	1.179
Control 2	-	84.5	93.7	42.8	1.095
0.15	0.15	92.2	86.9	44.1	1.209
0.33	0.27	90.8	87.3	41.6	1.024
0.73	0.64	88.3	90.5	43.6	1.145
1.7	1.5	89.9	87.0	41.9	1.024
3.5	3.2	94.1	77.5	43.2	1.087
7.9	7.3	0	-	-	-

Toxicity Observations: During the test, one fish in water control 1, two fish at 0.27 µg/L, and one fish at 1.5 µg/L exhibited spinal curvature.

Statistical Results:

Statistical Method: Cochran-Armitage trend test

NOEC: 1.5 µg/L benomyl **LOEC:** 3.2 µg/L benomyl **MATC:** 2.2 µg ai/L benomyl
Most sensitive endpoint: Larval survival.

Comments: Survival data for the exposure groups were compared to data for the pooled control.

11. REVIEWER'S STATISTICAL RESULTS:

Statistical Methods: Bonferroni's t-test
NOEC: 3.2 µg/L benomyl **LOEC:** 7.3 µg/L benomyl **MATC:** 4.8 µg/L benomyl
Most sensitive endpoint: Hatchability

Comments: Hatch, survival, and growth data from the exposure groups were compared to the data for the pooled control. The replicate means were used to analyze the growth, hatchability, and survival data.

12. REVIEWER'S COMMENTS: Although the individual growth data were not presented in the report, the reviewer accepts the conclusions of the authors. Upon visual examination of the means, length and wet weight showed no clear dose-response and appeared to be less sensitive endpoints than larval survival. This study is scientifically sound, meets the guideline requirements for a fish early life-stage study, and is classified as **Core**. Based on mean measured, calculated carbendazim concentrations, the MATC for exposed channel catfish was between 0.99 and 2.11 µg/L. The geometric mean MATC was 1.44 µg/L.

BASED ON THE RATIO OF BENOMYL MOLECULAR WEIGHT TO CARBENDAZIM MOLECULAR WEIGHT, nominal carbendazim concentrations of 0.0, 0.0 ,0.10. 0.22, 0.48, 1.10, 2.3, and 5.2 ug/L were converted by the study author to nominal benomyl equivalent concentrations of 0.0, 0.0, 0.15, 0.33, 0.73, 1.70, 3.50, and 7.90 ug/L using a conversion factor of 1.52. This conversion step is not necessary given that the test chemical is carbendazim, not benomyl. Agreement between the EPA and the registrant to use carbendazim instead of benomyl (due to rapid conversion of benomyl to carbendazim in water) was reached before study initiation. Therefore, study conclusions are reported as nominal CARBENDAZIM

concentrations as opposed to nominal benomyl concentrations.
(R. Petrie, EEB/EFED/OPP)