US EPA ARCHIVE DOCUMENT

5/9/1994

DP Barcode : D199854
PC Code No : 099101
EEB Out :

To: Linda Propst/Susanne Cerrelli, PM 73
Special Review and Reregisration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 099101
Chemical Name : Benomyl (Carbendazim)
Type Product : Fungicide
Product Name :
Company Name : DuPont
Purpose : Data reviews for 72-3a and 72-3b with carbendazim
Action Code : 606 Date Due : 06/28/94
Reviewer : William A. Erickson

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.
71-1(a)			72-2(a)			72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)	409750-01	S	122-1(a)		
71-2(b)			72-3(b)	409750-02	S	122-1(b)		
71-3			72-3(c)			122-2		
71-4(a)			72-3(d)			123-1(a)		
71-4(b)			72-3(e)			123-1(b)		
71-5(a)			72-3(f)			123-2		
71-5(b)			72-4(a)			124-1		
72-1(a)			72-4(b)			124-2		
72-1(b)			72-5			141-1		
72-1(c)			72-6			141-2		
72-1(d)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

1

5

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl: acute toxicity tests with carbendazim

FROM: for Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 3/29/94

TO: Linda Propst/Susanne Cerrelli PM 73
Special Review and Reregistration Division (7508W)

To support the data requirements for benomyl (carbendazim), E.I. du Pont de Nemours and Company, Newark, DE submitted the following studies:

Boeri, R.L. 1988. Static acute toxicity of carbendazim technical to the sheepshead minnow, *Cyprinodon variegatus*. Enseco Marblehead Laboratory Project DP1588, Enseco Inc., Marblehead, MA. MRID No. 409750-01.

Boeri, R.L. 1988. Flow through acute toxicity of carbendazim technical to the eastern oyster, *Crassostrea virginica*. Enseco Marblehead Laboratory Project DP2688, Enseco Inc., Marblehead, MA. MRID No. 409750-02.

Both studies have been classified as Supplemental; they do not fulfill guideline requirements but can be used in a risk assessment. Neither study established an LC_{50} value nor determined that it exceeded 100 ppm. Because anticipated residues in aquatic bodies are much lower than the NOELs determined in these studies, however, the studies do not need to be repeated unless the registrant wishes to do so or if application rates of benomyl are increased. Refer to the attached DERs for the classification and results of these studies.

If you have any questions, please contact Bill Erickson at 305-6212 or Henry Craven at 305-5320.

2

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6

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl (Carbendazim)
   Shaughnessey No.: 099101

2. **TEST MATERIAL:** Carbamic acid, 1H-benzimidazol-2-yl-,methyl ester; >98% purity; Haskell No. H-17,203. CAS # 10605-21-7.

3. **STUDY TYPE:** 72-3a. Acute toxicity to estuarine/marine fish.

4. **CITATION:** Boeri, R.L. 1988. Static acute toxicity of carbendazim technical to the sheepshead minnow, *Cyprinodon variegatus*. Enseco Marblehead Laboratory Project DP1588, Enseco Inc., Marblehead, MA. Submitted by E.I. du Pont de Nemours and Co., Newark, DE. MRID No. 409750-01.

5. **REVIEWED BY:**

   William A. Erickson
   Wildlife Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 3/25/94

6. **APPROVED BY:**

   Henry T. Craven
   Head, Section 4
   EEB/EFED

   Signature: Henry T. Craven
   Date: 3/25/94

7. **CONCLUSIONS:** The study is scientifically sound but does not fulfill the guideline requirement for an acute toxicity study for estuarine/marine fish. An LC_{50} value was not determined nor was it established to be >100 ppm. An LOEL was not determined; the NOEL was 1.158 ppm, the highest level tested.

8. **RECOMMENDATIONS:**

9. **BACKGROUND:**

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

11. Materials and Methods:

a. Test Animals

Used ASTM Standard Practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians (ASTM 1980).

Animal: recently hatched Cyprinodon variegatus
Source: commercial supplier
Acclimation: 30 to 50 days under test conditions; 14 days in 100% dilution water prior to test; recirculating system
Dilution water: 20 ppt salinity
Light: assumed photoperiod 14L:10D; light intensity unknown
Acclimation temperature: assumed to be 22 ± 1°C.
Food: during acclimation oyster were fed, but food type not reported; during test oysters not fed

b. Test System

Type: 96-hr static, nonrenewal; compound stable
Vessel Construction: glass aquaria
Vessel Size/Volume: 19 L with 10 L solution
Flow rate: none; static renewal test
Photoperiod: 14L:10D, cool-white fluorscent bulbs
Temperature: 22 ± 1°C
Aeration: none
Number of test concentrations: 5 plus diluent control and solvent control

c. Fish

10 fish/replicate; 2 replicates/concentration
Loading rate for fish: 0.14 gm/L
Age at test initiation: 30 to 50 days
Fish size: fish smaller in weight than recommended mean weight = 143.9 mg (range 44.5-347.8 mg); mean length = 21.2 mm (range 16-27 mm)

d. Test water

Source: natural Atlantic seawater from Marblehead MA (95 micron, carbon filtered)
Salinity: 20 ppt
pH: 7.5-7.9
Solvent: DMF at 0.5 mL/L
Controls: concurrent water and solvent controls used

e. Test Food: not fed

f. Test Concentrations: in µg/L

Range Finding Test: used to determine final conc.
Nominal: 196, 265, 441, 735, 1225
Measured: 196, 246, 388, 684, 1158
Method of Measurement: HPLC

g. Test Duration: 4 days

h. Toxic indicators: survival, loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, curved spine, hemorraghing, change in behavior

i. Other Parameters Measured (all test concentrations):

Daily: salinity (20 ppt), dissolved oxygen (4.3-8.2), pH (7.5-7.9) and temperature (22°C). Test not repeated due to low oxygen because there was 100% survival.

j. Statistical analysis

Not needed because 100% survival in all concentrations.

12. REPORTED RESULTS:

a. Data

Raw data included.

b. Analysis of Test Concentrations

Chemical analyses of the test concentrations were performed. Results are reported by nominal and measured concentrations (measured concentrations were usually within $\geq$ 88% of the nominal concentrations).

c. Reported Results

1. NOEL AND LOEL: none reported; NOEL > 1,158 µg/L (measured concentration)

2. Survival: 100% for controls; 100% for all test concentrations

3. Sublethal Effects: none observed

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A Good Laboratory Practice Statement was included with the report.

5
9

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

a. Test Procedure.

This study was performed under conditions that seem to comply with 1986 techniques and are reasonably consistent with current Guideline standards.

b. Statistical Analysis.

Statistics were not conducted on the data because there was no mortality

c. Results/Discussion.

The NOEL is > 1,158 µg/L (measured concentrations).

d. Adequacy of the Study:

(1) Classification: Supplemental

(2) Rationale: An LC_{50} value was not determined nor was it established to exceed 100 ppm. The data can be used for a risk assessment if the LC_{50} value for technical carbendazim is assumed to be 1.158 ppm and aquatic residues do not exceed this level (i.e., present application rate is not raised). Alternatively, the registrant may wish to repeat the test to determine an LC_{50} value or establish that it exceeds 100 ppm.

(3) Repairability: No

15. COMPLETION OF ONE-LINER: Yes.

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl (Carbendazim)
   Shaughnessey No.: 099101

2. **TEST MATERIAL:** Carbamic acid, 1H-benzimidazol-2-yl-,methyl ester; >98% purity; Haskell No. H-17,203. CAS # 10605-21-7.

3. **STUDY TYPE:** 72-3b. Acute toxicity to estuarine/marine mollusk.

4. **CITATION:** Boeri, R.L. 1988. Flow through acute toxicity of carbendazim technical to the eastern oyster, *Crassostrea virginica*. Enseco Marblehead Laboratory Project DP2688, Enseco Inc., Marblehead, MA. Submitted by E.I. du Pont de Nemours and Co., Newark, DE. MRID No. 409750-02.

5. **REVIEWED BY:**

   William A. Erickson
   Wildlife Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 3/25/94

6. **APPROVED BY:**

   Henry T. Craven
   Head, Section 4
   EEB/EFED

   Signature: Henry T. Craven
   Date: 3/25/94

7. **CONCLUSIONS:** The study is scientifically sound but does not fulfill the guideline requirement for an acute toxicity study for estuarine/marine mollusk. An LC_{50} value was not determined nor was it established to be >100 ppm. An LOEL was not determined; the NOEL was 1.145 ppm, the highest level tested.

8. **RECOMMENDATIONS:**

9. **BACKGROUND:**

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

11. Materials and Methods:

a. Test Animals

Used ASTM Standard Practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians (ASTM 1980).

Animal: young Crassostrea virginica
Source: commercial supplier
Acclimation: at least 10 days under test conditions; at least 10 days in 100% dilution water prior to test; recirculating system
Dilution water: 30 ppt salinity
Light: 16L:8D photoperiod; light intensity unknown
Acclimation temperature: not reported assumed to be about 20°C
Food: during acclimation and test; oysters exposed to unfiltered sea water, no supplemental feeding

b. Test System

Type: 96-hr flow-through using Brungs-Mount diluter
Vessel Construction: glass aquaria
Vessel Size/Volume: 38 L divided into 2 separate chambers, each 19 L size containing 10 L solution
Flow rate: approximately 0.58 L/oyster/hr
Photoperiod: 16L:8D, cool-white fluorscent bulbs
Temperature: 20.0°C
Aeration: not needed
Number of test concentrations: 5 plus diluent control and solvent control

c. Oyster

10 oysters/replicate; 2 replicates/concentration
Loading rate: N\A
Age at test initiation: unknown
Size: 25-50 mm height

d. Test water

Source: unfiltered natural Atlantic seawater from Marblehead MA;
Salinity: 30 ppt
pH: 7.6-7.9
Solvent: DMF at 0.5 mL/L
Controls: concurrent water and solvent controls used

e. Test Food: unfiltered sea water; not supplementally fed

f. Test Concentrations: in µg/L

8
12

Range Finding Test: not used for oyster; used data for mysid and sheepshead minnow
Nominal: 135, 243, 431, 728, 1213
Measured: 89, 212, 358, 652, 1145
Method of Measurement: HPLC

g. Test Duration: 4 days

h. Toxic indicators: new shell deposition, survival, lack of feces production, appearance of mucus, loss of reflex

i. Other Parameters Measured (all test concentrations):

Daily: salinity (30 ppt), dissolved oxygen (6.3-7.9), pH (7.6-7.9) and temperature near 20°C (mean = 19.5°C; range = 18.3-20.0°C). Lowest temperatures occurred at 72 hrs in all containers, therefore temperature effect was the same among all treatments and should not affect outcome of this study.

j. Statistical analysis

Not needed because survival $\geq$ 90% for 96 hrs in all concentrations; mean shell deposition ranged from 1.55 to 1.70 mm; overall mean 1.61 mm

k. Other Data

96-hr acute data for mysids given as 98 µg/L

12. REPORTED RESULTS:

a. Data

Raw data included.

b. Analysis of Test Concentrations

Chemical analyses of the test concentrations were performed. Results are reported by nominal and measured concentrations (measured concentrations were usually within $\geq$ 83% of the nominal concentrations except for lowest concentration where measured concentration was 66% of nominal).

c. Reported Results

1. NOEL AND LOEL: none reported; NOEL > 1,145 µg/L (measured concentration). Mean shell deposition varied from 97 to 106% of the controls (no statistically significant differences noted among controls and test concentrations);

2. Survival: 100% in dilution water control; 90% in solvent control; ≥ 95% for all test concentrations

3. Sublethal Effects: none observed

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A Good Laboratory Practice Statement was included with the report.

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

a. Test Procedure.

This study was performed under conditions that seem to comply with techniques and are reasonably consistent with current Guideline standards.

b. Statistical Analysis.

Descriptive statistics indicated that there were not effects; other statistical tests not conducted.

c. Results/Discussion.

The NOEL is > 1,145 μg/L (measured concentrations).

d. Adequacy of the Study:

(1) Classification: Supplemental

(2) Rationale: An LC_{50} value was not determined nor was it established to exceed 100 ppm. The data can be used for a risk assessment if the LC_{50} value for technical carbendazim is assumed to be 1.145 ppm and aquatic residues do not exceed this level (i.e., present application rate is not raised). Alternatively, the registrant may wish to repeat the test to determine an LC_{50} value or establish that it exceeds 100 ppm.

(3) Repairability: No

15. COMPLETION OF ONE-LINER: Yes.

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl: daphnid and mysid acute toxicity tests

FROM: Anthony F. Maciorowski, Chief H.T. Craven 2/18/94
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Susanne Cerrelli
Reregistration Branch
Special Review and Reregistration Division (7508W)

E.I. du Pont de Nemours and Company, Newark, DE submitted the following studies to support reregistration of benomyl:

Baer, K.N. 1992. Static, acute, 48-hour EC_{50} of DPX-E965-299 (Carbendazim, MBC) to *Daphnia magna*. Conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE. MRID No. 424142-01.

Ward, T.J. and R.L. Boeri. 1992. Acute flow through toxicity of DPX-T1991 (Benlate 50 WP); H17201 to the mysid, *Mysidopsis bahia*. Conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE. MRID No. 424142-02.

These studies are scientifically sound and fulfill the guideline requirements for 72-2a (MBC) and 72-3f. Refer to the enclosed Data Evaluation Records for the results and classification of these tests.

If you have any questions, please contact Bill Erickson at 305-6212 or Henry Craven at 305-5320.

11

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DP Barcode : D181429
PC Code No : 099101
EEB Out :

To: Susanne Cerrelli
Reregistration Branch, SRRD (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 099101
Chemical Name : Benomyl
Type Product : Fungicide
Product Name :
Company Name : DuPont
Purpose : Reregistration data

Action Code : 627 Date Due : 11/08/92
Reviewer : William A. Erickson

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.
71-1(a)			72-2(a)	424142-01	Y	72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)			122-1(a)		
71-2(b)			72-3(b)			122-1(b)		
71-3			72-3(c)			122-2		
71-4(a)			72-3(d)			123-1(a)		
71-4(b)			72-3(e)			123-1(b)		
71-5(a)			72-3(f)	424142-02	Y	123-2		
71-5(b)			72-4(a)			124-1		
72-1(a)			72-4(b)			124-2		
72-1(b)			72-5			141-1		
72-1(c)			72-6			141-2		
72-1(d)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

12

16

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl (Carbendazim, MBC). Shaughnessey No. 099101.

2. **TEST MATERIAL:** Carbic acid, 1 H-benzimidazol-2-yl-, methyl ester (DPX-E965-299); Lot F00701B; CAS No. 10605-21-7; 99.3% active ingredient.

3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Baer, K.N. 1992. Static, Acute, 48-Hour EC_{50} of DPX-E965-299 (Carbendazim, MBC) to *Daphnia magna*. Report No. 185-92. Prepared and submitted by E.I. du Pont de Nemours and Co., Inc., Newark, DE. EPA MRID No. 424142-01.

5. **REVIEWED BY:**

Carolyn F. Poppell, Sc.M.
Senior Scientist
KBN Engineering and Applied Sciences, Inc.

Signature: Louise McRae for CFP
Date: 10/13/93

6. **APPROVED BY:**

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

Signature: Rosemary Graham Mora
Date: 10/12/93

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

Signature: W. Graham, 2/18/94
Date: Henry T. Craven 2/18/94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using a freshwater invertebrate. With a 48-hour EC_{50} of 0.39 mg/l mean measured concentration, DPX-E965-299 is classified as highly toxic to *Daphnia magna*. The NOEC was 0.11 mg/l mean measured concentration.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** First instar *Daphnia magna* (<24 hours old) were obtained from in-house cultures of 13 to 22 day old parent daphnids. Daphnids were cultured in 1-l Pyrex beakers containing 0.9 l of filtered dilution water maintained at 20°C. Daphnids were fed two green algal species three times weekly. Daphnids were not observed for sickness, injury, or abnormality.

B. **Test System:** The test was conducted under static conditions in 250-ml Pyrex beakers containing 200 ml of test solution. Test solutions were maintained between 20.3 and 21.0°C. The photoperiod was 16 hours of light (96-129 lux) with a 25-minute transition period.

The dilution (culture) water was laboratory well water which flowed through aquaria containing fathead minnows. At test initiation, the dilution water had a conductivity of 190 µmhos/cm, and a hardness and alkalinity of 80 and 79 mg/l as $CaCO_3$, respectively.

A stock solution (2 mg/ml) was prepared by dissolving test material in dimethylformamide (DMF) and used immediately.

C. **Dosage:** Forty-eight-hour static test. Based on previous testing, six nominal concentrations (0.084, 0.12, 0.17, 0.25, 0.35, and 0.50 mg/l) were selected for this study. A solvent (DMF) control and a dilution water control were also included in the study.

D. **Design:** The test consisted of four replicates per test concentration and control. Five daphnids were randomly placed in each replicate. Daphnids were not fed during the test.

Daily observations were made for immobility. Dissolved oxygen concentration (DO) and pH were measured in all replicates before daphnids were added to the test chambers and at test termination. Temperature was measured daily in all replicates.

The concentration of active ingredient in the test solutions was determined by high performance liquid chromatography. Solutions were analyzed from aliquots taken on days 0 and 2 of the study.

E. **Statistics:** The 48-hour median effect concentration (EC_{50}) and 95% confidence interval were determined by

14

probit analysis. The NOEC was determined by analysis of variance.

12. **REPORTED RESULTS:** Mean measured concentrations were 0.075, 0.11, 0.16, 0.29, 0.41, and 0.56 mg/l (Appendix I, Table I, attached).

No immobility was observed in the control, solvent control, or the two lowest test levels (0.075 and 0.11 mg/l) (Table 4, attached). Immobilization of 30-85% was reported at a concentrations ≥0.16 mg/l. The 48-hour EC_{50} was 0.39 mg/l, with a 95% confidence interval of 0.27 to 0.75 mg/l (Table 5, attached). The slope of the dose-response curve was 5.1. The no-observed-effect concentration (NOEC), based on absence of immobility after 48 hours, was 0.11 mg/l.

During the test, DO ranged from 9.1 to 9.2 mg/l and pH ranged from 8.0 to 8.6. The temperature ranged from 20.3 to 21.0°C (mean of 20.6°C).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** DPX-E965-299 was highly toxic to *Daphnia magna* neonates in an unaerated, static 48-hour test.

Quality Assurance and Study Compliance Statements were included in the report, indicating that the study was conducted in accordance with USEPA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP except for the following:

The concentration of DMF in the solvent control was not reported; the SEP recommends a solvent concentration of ≤0.5 ml/l under static conditions.

The author did not describe the physical characteristics of the test material (i.e., color and physical state).

The author did not report observations of death, disease, or stress in the brood population during the 48 hours prior to study initiation.

The method used to maintain the test temperature was not reported and temperature measurements were made on a daily basis. The guidelines state that temperature should be measured every six hours if temperature is

15
F9

controlled by a water bath, otherwise hourly measurements should be made.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the EC_{50}. Using the moving average method, Toxanal estimated an EC_{50} of approximately 0.48 with 95% confidence limits of 0.38 and 0.95 mg/l (see attached printout). The reviewer's statistical analysis yielded a slightly higher EC_{50} than that reported by the author. The EC_{50} derived by the author using the probit method is lower (more conservative) and is therefore accepted.

C. **Discussion/Results:** The deviations listed above probably did not affect the test results. This study is scientifically sound and meets the guideline requirements for an acute toxicity test using *Daphnia magna*. With a 48-hour EC_{50} was 0.39 mg/l mean measured concentration, DPX -E965-299 is classified as highly toxic to *Daphnia magna*. The NOEC was 0.11 mg/l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes. September 21, 1992.

16

EEB Review dated 5/9/1994 Benomyl

Page _____ is not included in this copy.

Pages 17 through 19 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidentia by product registrants. If you have any questions, please contac the individual who prepared the response to your request.

Carolyn Poppell Benomyl Daphnia magna 09-21-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.56	20	16	80	.5908966
.41	20	4	20	.5908966
.29	20	8	40	25.17223
.16	20	8	40	25.17223
.11	20	0	0	9.536742E-05
.075	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .11 AND .56 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .4791659

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.5837123	.4791658	.3837313	.9490941

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.566746	5.256401	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.507856
95 PERCENT CONFIDENCE LIMITS =-.6312203 AND 5.646933

LC50 = .397915
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .1239886
95 PERCENT CONFIDENCE LIMITS = 0 AND .2716915

****

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl (Carbendazim MBC). Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® 50 WP; Lot F60113G; CAS No. (benomyl, the active ingredient) 17804-35-2; 50% active ingredient; a wettable powder.

3. **STUDY TYPE:** 72-3. Estuarine Shrimp Flow-Through Acute Toxicity Test. Species Tested: Mysid (*Mysidopsis bahia*).

4. **CITATION:** Ward, T.J. and R.L. Boeri. 1992. Acute Flow Through Toxicity of DPX-T1991 (Benlate® 50 WP); H17201 to the Mysid, *Mysidopsis bahia*. Report No. 253-92. Prepared by Resource Analysts, Inc., Hampton, NH. Submitted by E.I. du Pont de Nemours and Co., Inc., Newark, DE. EPA MRID No. 424142-02.

5. **REVIEWED BY:**

Carolyn F. Poppell, Sc.M.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Reffer for CFP
Date: 10/13/92

6. **APPROVED BY:**

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

Signature: Rosemary Graham Mora
Date: 10/12/92

W. Linhart 3/18/94

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

Signature: Henry T. Craven
Date: 2/18/94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a flow-through acute toxicity test using estuarine/marine shrimp. With a 96-hour LC_{50} of 140 µg a.i./l mean measured concentration, Benlate® (a formulated product) is classified as highly toxic to *Mysidopsis bahia*. The NOEC is 100 µg a.i./l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile mysids (*Mysidopsis bahia*), less than 24 hours old, were obtained from in-house cultures. Mysid cultures were acclimated to test conditions for at least 14 days before juveniles were collected for testing. The temperature of the culture was maintained at 19.4-20.4°C. The adult mysids showed no signs of disease or abnormalities during the acclimation period.

B. **Test System:** A proportional diluter was used to prepare and deliver the test solutions. A stock solution of test substance (2 g/l) was prepared in dimethylformamide (DMF). Appropriate amounts of the stock solution were added directly to dilution water by a proportional diluter and mixed by a high shear pump. Test vessels were 20-l glass aquaria filled with 15 l of test solution or control dilution water. The depth of test solution in each test chamber was approximately 18 cm. The diluter was calibrated before and after the test. The flow rate from the diluter was sufficient to provide 6.9 media exchanges per day in the aquaria.

The laboratory environment was maintained on a 16-hour daylight photoperiod with a 15-minute transition period between light and dark. The light intensity during the test was approximately 10 $uEs^{-1}m^{-2}$. Test vessels were randomly placed in a temperature-controlled water bath set at 22 ±1°C.

Natural seawater, collected from the Atlantic Ocean, was adjusted to a salinity of 11 to 17 parts per thousand (ppt) and stored in 500-gallon polyethylene tanks where it was aerated before use as dilution water. At test initiation, the dilution water had a pH range of 7.9-8.0 and a salinity of 17 ppt.

C. **Dosage:** Ninety-six-hour test. Based on the results of range-finding tests, five nominal concentrations (70, 125, 200, 300 and 500 µg a.i./l) were selected for this study. A solvent control (0.5 ml DMF/l) and a dilution water control were also included.

D. **Design:** Ten mysids were impartially selected and placed in glass/screen cages within each aquarium. Two replicates were used for each treatment level, for a total of 20 organisms per concentration. The mysids were fed live brine shrimp nauplii daily during the

22
~~21~~

test. The loading rate of control mysids during the study was approximately 0.33 mg/l.

Observations of mortality and treatment-related effects were made at 24, 48, 72, and 96 hours. Dissolved oxygen concentration (DO), pH, salinity, and temperature were measured and recorded daily in each test chamber that contained live animals. The temperature in one test vessel was recorded continuously during the test.

Water samples from each test chamber were taken at test initiation and termination to verify concentrations of active ingredient in solution. The concentration of Benomyl was determined using high performance liquid chromatography.

E. **Statistics:** The 96-hour median lethal concentration (LC_{50}) was estimated using the moving average method, according to statistical techniques of Stephan (1983).

12. **REPORTED RESULTS:** The mean measured concentrations were 62, 100, 160, 240, and 400 µg a.i./l (Table 2, attached).

There were no mortalities in the dilution water or solvent control groups, and all mysids appeared normal throughout the test period (Table 3, attached). The 96-hour LC_{50} was 140 µg a.i./l, with 95% confidence limits of 110 to 170 µg/l. The 96-hour no-observed effect concentration (NOEC) was estimated to be 100 µg/l.

During the test, DO ranged from 7.0 to 8.7 mg/l, pH values ranged from 7.7 to 8.2, and temperature ranged from 21.6 to 22.8°C. The salinity range was 15-17 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors presented no conclusions.

Quality Assurance and Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

23
28

The temperature during acclimation (19.4 to 20.4°C) was lower than the temperature maintained during the test (21.6 to 22.0°C).

The authors stated that the temperature in one test vessel was monitored continuously during the test, however, the measurements were not reported.

The test organisms were impartially distributed to the test chambers; random assignment to the test vessels is required.

The test was conducted using a formulated test material; the test design should have included a control using the inert or carrier ingredients.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the median lethal concentration (LC_{50}) and 95% confidence interval and obtained results similar to those of the authors (see attached printout).

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a flow-through acute saltwater shrimp toxicity study. The 96-hour LC_{50} was 140 µg a.i./l mean measured concentration. This classifies Benlate® (a formulated product) as highly toxic to *Mysidopsis bahia*. The NOEC was 100 µg a.i./l. The single mortality at the 62 µg/l treatment level does not appear to be treatment-related.

D. **Adequacy of the Study:**

(1) Classification: Core for a formulated product.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes. September 29, 1992.

24
29

EEB Review dated 5/9/1994 Benomyl

Page _____ is not included in this copy.

Pages 25 through 26 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Carolyn Poppell Benlate 50 WP Mysids 09-29-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
400	20	20	100	9.536742E-05
240	20	20	100	9.536742E-05
160	20	14	70	5.765915
100	20	0	0	9.536742E-05
62	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 100 AND 240 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 143.9677

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	6.572957E-02	140.9016	118.4365	165.1228

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	2.535007	7.095332	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 7.989051

95 PERCENT CONFIDENCE LIMITS =-4.730881 AND 20.70898

LC50 = 139.0264

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 96.41168

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****

DP Barcode : D187285
PC Code No : 099101
EEB Out :FEB 15 1994

To: Linda Propst, PM 73
Special Review and Reregisration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 099101
Chemical Name : Benomyl
Type Product : Fungicide
Product Name :
Company Name : E.I. du Pont de Nemours and Company
Purpose : Reregistration data

Action Code : 627 Date Due : 04/27/93
Reviewer : William A. Erickson

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.
71-1(a)			72-2(a)			72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)			122-1(a)		
71-2(b)			72-3(b)			122-1(b)		
71-3			72-3(c)			122-2		
71-4(a)			72-3(d)	426264-02	Y	123-1(a)		
71-4(b)			72-3(e)	426264-01	Y	123-1(b)		
71-5(a)			72-3(f)			123-2		
71-5(b)			72-4(a)			124-1		
72-1(a)			72-4(b)	425294-01	Y	124-2		
72-1(b)			72-5			141-1		
72-1(c)			72-6			141-2		
72-1(d)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

28
33

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

FEB 15 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl: review of reregistration data

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Linda Propst, PM 73
Special Review and Reregistration Division (7508W)

E.I. du Pont de Nemours and Company, Newark, DE submitted the following studies to support reregistration of benomyl:

Graves, W.C. and J.P. Swigert. 1993. Benlate fungicide 50 DF: a 96-hour shell deposition test with the eastern oyster (*Crassostrea virinica*). Conducted by Wildlife International Ltd., Easton, MD. MRID No. 426264-01.

Graves, W.C. and J.P. Swigert. 1993. Benlate fungicide 50 DF: a 96-hour static acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*). Conducted by Wildlife International Ltd., Easton, MD. MRID No. 426264-02.

Baer, K.N. 1992. Chronic toxicity of DPX-E965-299 (carbendazim, MBC) to *Daphnia magna*. Conducted by Haskell Laboratory for Toxicology and Industrial Medicine. MRID No. 425294-01.

The three studies are scientifically sound and fulfill the guideline requirements for 72-3d, 72-3e, and 72-4b. Refer to the enclosed Data Evaluation Records for the results and classification of each study.

If you have any questions, please contact Bill Erickson at 305-6212 or Henry Craven at 305-5320.

Recycled/Recyclable
Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

29
34

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® Fungicide 50 DF; [1-[(butylamino) carbonyl]-1H-benzimidazol-2-yl]-carbamic acid, methyl ester; CAS No. 17804-35-2 for benomyl; Batch No. 1210870300; 50.0% active ingredient; a tan powder.

3. **STUDY TYPE:** 72-3. Mollusc 96-Hour Flow-Through Shell Deposition Study. Species Tested: eastern oyster (*Crassostrea virginica*).

4. **CITATION:** Graves, W.C. and J.P. Swigert. 1993. Benlate® Fungicide 50 DF: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*). Project No. 112A-109. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Submitted by E.I. du Pont de Nemours and Company, Newark, DE. EPA MRID No. 426264-01.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 8/2/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 3/2/93

W. Erichsen, 2/07/94

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

Signature: Henry Craven
Date: 2/15/94

Woodyard 2/16/94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using eastern oysters. The 96-hour EC_{50} value of 78 µg/l (mean measured concentration of Benlate® 50 DF) classifies Benlate® 50 DF as very highly toxic to eastern oysters. The NOEC could not be determined due to shell growth inhibition at all test levels.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier in Pasadena, MD. The oysters were held under test conditions for at least 10 days prior to test initiation. During the holding period, the oysters appeared healthy. They were supplied with unfiltered natural seawater and supplemented with additions of algae. During holding, the temperature was 21.2-22.7°C, the salinity was 25 parts per thousand (ppt), the pH was 7.7-8.1, and the dissolved oxygen concentration (DO) was 5.6-6.8 mg/l.

An indiscriminately selected sample of 20 oysters had an average length of 27 mm (25-31 mm). Immediately prior to test initiation, 3-7 mm of shell periphery was removed from each oyster using a motorized grinder.

B. **Test System:** A continuous-flow diluter was used. The diluter was preconditioned with the test material for approximately 5 hours prior to testing. Each test chamber received approximately 1 l of test solution per oyster per hour. The test chambers were Teflon®-lined, 56-l polyethylene aquaria filled with 12.6 l of test solution. The test solution depth was approximately 7 cm. The aquaria were impartially positioned in a temperature-controlled water bath designed to maintain 22 ±1°C. The laboratory environment was maintained on a 16-hour daylight photoperiod with a light intensity of approximately 291 lux. Thirty-minute dawn and dusk simulations were used.

Unfiltered natural seawater, collected at Indian River Inlet, DE, was aerated and diluted with well water before use as test dilution water. The salinity of the dilution water was 25-27 ppt and the pH was 7.7-7.8 during the 4-week period immediately preceding the test.

One stock solution was prepared for each of the five concentrations. The first stock (4.18 mg/ml) was prepared by dissolving the test material in dimethylformamide (DMF). Aliquots of this stock were diluted with DMF to prepare the four additional stocks. The individual stocks were delivered to the diluter mixing chambers.

C. **Dosage:** Ninety-six-hour acute flow-through toxicity test. Five concentrations (78, 130, 220, 360, and 600 µg/l) a solvent control, and a dilution water control were used. The concentration of DMF in the solvent control and treatment groups was 0.14 ml/l.

D. **Design:** Twenty oysters were impartially selected and distributed to each aquarium, one aquarium per concentration. To supplement the oyster diet, an algal suspension (*Thalassiosira* sp. and *Skeletonema* sp.) was added to the system.

Observations of mortality and clinical signs of toxicity were made daily. At the end of the test, the length of the longest finger of new shell growth on each oyster was measured to the nearest 0.05 mm. Shell growth inhibition in each treatment group was expressed as a percentage of the mean growth in the controls.

The DO, salinity, and pH were measured in each test chamber on days 0, 2, and 4. The temperature was measured in each chamber at the beginning and end of the test. The temperature of the dilution water control vessel was recorded continuously.

Test solution samples were collected from each test chamber at 0, 24, 48, 72, and 96 hours. The samples were shipped on ice to the sponsor for analysis using high performance liquid chromatography. The concentration of whole test material was calculated from measured concentrations of carbendazim (the aqueous degradation product of Benomyl).

E. **Statistics:** Shell deposition measured in the dilution water control and solvent control were compared by a t-test and found to be significantly different. Shell growth inhibition in each treatment group was expressed as a percentage of the mean growth of the solvent control. The 96-hour EC_{50} value and 95% confidence interval were determined using the percent inhibition data and the probit method.

12. **REPORTED RESULTS:** Analytical data for water samples were presented in Table 1 (attached). The mean measured concentrations for the test, based on measured carbendazim and presented as µg/l of Benlate® 50 DF, were 62, 110, 155, 277, and 444 µg/l.

There were no mortalities during the test. Oyster shell growth in the dilution water control and solvent control

were significantly different and averaged 5.32 and 4.30 mm, respectively (Table 3, attached). "The presence of the solvent likely did not contribute to the observed toxicity of the test substance." The length measurements indicated shell growth inhibition ranging from 42.8% in the 62 µg/l group to 99.8% in the 444 µg/l (Table 3, attached).

During the test, the DO ranged from 6.7 to 7.4 mg/l (>60% of saturation). The pH values ranged from 7.7 to 7.9 and the salinity was 24-25 ppt. Based on individual measurements, the temperature was 21.6-21.9°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour EC_{50} value was 78 µg/l (95% C.I. = 62-110 µg/l) of formulation. Based on the inhibition in shell growth observed at all exposure concentrations, the no-observed-effect concentration (NOEC) was <62 µg/l.

Good Laboratory Practice Compliance and Quality Assurance Statements were included in the report indicating compliance to with EPA Good Laboratory Practice Standards under the Federal Insecticide, Fungicide, and Rodenticide Act.

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

A. **Test Procedure:** The test procedures were generally in accordance with the guidelines with the following deviations:

The test material was a formulated product. This study should have included a formulation control containing the highest concentration of inert or carrier ingredients present in the highest test level.

In this study, the flow rate of the test solution was 1 l/oyster/hour. According to the protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976), each oyster should receive a minimum of 5 l of flow-through test solution per hour. However, this study is acceptable since a supplemental food was added and the control oysters had an adequate growth during the test.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to determine the 96-hour EC_{50} value as 78 µg/l (95% C.I. = 67-88 µg/l). The slope of the concentration response curve was 3.0 (see attached printout 1). The reviewer used Steel's Many-One rank test (Toxstat version 3.3) to determine the NOEC (see attached printout 2). The growth of oysters at all

test levels was significantly reduced when compared to that of the solvent control.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using eastern oysters. The 96-hour EC_{50} value of 78 µg/l (mean measured concentration of Benlate® 50 DF) classifies Benlate® 50 DF as very highly toxic to eastern oysters. The NOEC could not be determined due to shell growth inhibition at all test levels.

D. **Adequacy of the Study:**

(1) Classification: Core for a formulated product.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 02-12-93.

EEB Review dated 5/9/1994 Benomyl

Page _____ is not included in this copy.

Pages 35 through 37 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidentia by product registrants. If you have any questions, please contac the individual who prepared the response to your request.

#1

RIFICI BENLATE 50 DF EASTERN OYSTER 02-12-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
444	100	100	100	0
277	100	95	95	0
155	100	80	80	0
110	100	61	61	0
62	100	43	43	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 77.43546

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.5942988	77.43546	48.77767	101.8042

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	3.790392E-02	1	.2695764

SLOPE = 2.962409

95 PERCENT CONFIDENCE LIMITS = 2.38566 AND 3.539159

LC50 = 78.27856

95 PERCENT CONFIDENCE LIMITS = 67.10738 AND 88.39518

LC10 = 29.17008

95 PERCENT CONFIDENCE LIMITS = 20.37801 AND 37.3979

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38
43

426264-01, BENLATE 50 DF, OYSTER SHELL DEPOSITION
File: A:42626401.OYS Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	4.3025	CALCULATED t VALUE =	-2.4557
GRP2 (BLANK CRTL) MEAN =	5.3200	DEGREES OF FREEDOM =	38
DIFFERENCE IN MEANS =	-1.0175		

TABLE t VALUE (0.05 (2),40) = 2.021** SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	SOLVENT CONTROL	4.302				
2	DILUTION CONTRL	5.320	495.50	325.00	20.00	
3	62 µG/L	2.460	241.00	325.00	20.00	*
4	110	1.690	219.00	325.00	20.00	*
5	150	0.873	210.00	325.00	20.00	*
6	277	0.203	210.00	325.00	20.00	*
7	444	0.013	210.00	325.00	20.00	*

Critical values use k = 6, are 1 tailed, and alpha = 0.05

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	20	2.450	7.250	4.302
2	DILUTION CONTRL	20	3.000	7.600	5.320
3	62 µG/L	20	1.300	3.900	2.460
4	110	20	0.000	3.050	1.690
5	150	20	0.000	1.900	0.873
6	277	20	0.000	1.250	0.203
7	444	20	0.000	0.250	0.013

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	1.685	1.298	0.290
2	DILUTION CONTRL	1.930	1.389	0.311
3	62 µG/L	0.470	0.686	0.153
4	110	0.610	0.781	0.175
5	150	0.356	0.597	0.133
6	277	0.173	0.416	0.093
7	444	0.003	0.056	0.013

TITLE: 426264-01, BENLATE 50 DF, OYSTER SHELL DEPOSITION
FILE: A:42626401.OYS
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	4.0000	4.0000
1	SOLVENT CONTROL	2	4.0000	4.0000
1	SOLVENT CONTROL	3	5.8500	5.8500
1	SOLVENT CONTROL	4	4.1500	4.1500
1	SOLVENT CONTROL	5	2.8000	2.8000
1	SOLVENT CONTROL	6	2.9500	2.9500
1	SOLVENT CONTROL	7	2.4500	2.4500
1	SOLVENT CONTROL	8	4.9000	4.9000
1	SOLVENT CONTROL	9	4.0500	4.0500
1	SOLVENT CONTROL	10	4.0000	4.0000
1	SOLVENT CONTROL	11	3.6000	3.6000
1	SOLVENT CONTROL	12	5.8500	5.8500
1	SOLVENT CONTROL	13	3.2500	3.2500
1	SOLVENT CONTROL	14	4.6000	4.6000
1	SOLVENT CONTROL	15	6.6000	6.6000
1	SOLVENT CONTROL	16	4.7500	4.7500
1	SOLVENT CONTROL	17	4.7500	4.7500
1	SOLVENT CONTROL	18	3.4000	3.4000
1	SOLVENT CONTROL	19	7.2500	7.2500
1	SOLVENT CONTROL	20	2.8500	2.8500
2	DILUTION CONTRL	1	4.7500	4.7500
2	DILUTION CONTRL	2	5.2500	5.2500
2	DILUTION CONTRL	3	6.2500	6.2500
2	DILUTION CONTRL	4	7.6000	7.6000
2	DILUTION CONTRL	5	3.5500	3.5500
2	DILUTION CONTRL	6	5.9500	5.9500
2	DILUTION CONTRL	7	6.6000	6.6000
2	DILUTION CONTRL	8	7.0000	7.0000
2	DILUTION CONTRL	9	4.1500	4.1500
2	DILUTION CONTRL	10	3.0000	3.0000
2	DILUTION CONTRL	11	5.0500	5.0500
2	DILUTION CONTRL	12	3.3500	3.3500
2	DILUTION CONTRL	13	3.1000	3.1000
2	DILUTION CONTRL	14	5.1000	5.1000
2	DILUTION CONTRL	15	5.8000	5.8000
2	DILUTION CONTRL	16	5.8500	5.8500
2	DILUTION CONTRL	17	4.6000	4.6000
2	DILUTION CONTRL	18	5.5000	5.5000
2	DILUTION CONTRL	19	6.8000	6.8000
2	DILUTION CONTRL	20	7.1500	7.1500
3	62 µG/L	1	2.9000	2.9000
3	62 µG/L	2	1.3000	1.3000
3	62 µG/L	3	3.3000	3.3000
3	62 µG/L	4	2.2500	2.2500
3	62 µG/L	5	2.1500	2.1500
3	62 µG/L	6	2.6000	2.6000
3	62 µG/L	7	1.9000	1.9000
3	62 µG/L	8	2.2000	2.2000
3	62 µG/L	9	1.8500	1.8500
3	62 µG/L	10	1.9500	1.9500
3	62 µG/L	11	1.4000	1.4000
3	62 µG/L	12	2.5000	2.5000
3	62 µG/L	13	2.6500	2.6500
3	62 µG/L	14	2.2000	2.2000
3	62 µG/L	15	2.6000	2.6000
3	62 µG/L	16	3.9000	3.9000
3	62 µG/L	17	2.0000	2.0000
3	62 µG/L	18	3.2500	3.2500
3	62 µG/L	19	3.6000	3.6000
3	62 µG/L	20	2.7000	2.7000
4	110	1	2.0500	2.0500
4	110	2	2.2000	2.2000

4	110	3	1.1500	1.1500
4	110	4	2.2000	2.2000
4	110	5	1.0000	1.0000
4	110	6	0.0000	0.0000
4	110	7	1.8500	1.8500
4	110	8	0.8000	0.8000
4	110	9	1.5000	1.5000
4	110	10	3.0500	3.0500
4	110	11	1.1500	1.1500
4	110	12	2.5500	2.5500
4	110	13	0.7000	0.7000
4	110	14	1.2000	1.2000
4	110	15	2.6000	2.6000
4	110	16	1.9000	1.9000
4	110	17	1.7000	1.7000
4	110	18	1.5500	1.5500
4	110	19	2.9000	2.9000
4	110	20	1.7500	1.7500
5	150	1	0.8000	0.8000
5	150	2	1.9000	1.9000
5	150	3	0.0000	0.0000
5	150	4	0.9000	0.9000
5	150	5	0.3000	0.3000
5	150	6	0.6500	0.6500
5	150	7	1.8500	1.8500
5	150	8	1.6500	1.6500
5	150	9	0.9000	0.9000
5	150	10	0.0000	0.0000
5	150	11	1.2000	1.2000
5	150	12	1.3500	1.3500
5	150	13	0.6500	0.6500
5	150	14	1.0000	1.0000
5	150	15	1.2000	1.2000
5	150	16	0.7500	0.7500
5	150	17	1.2500	1.2500
5	150	18	0.0000	0.0000
5	150	19	0.0000	0.0000
5	150	20	1.1000	1.1000
6	277	1	0.0000	0.0000
6	277	2	0.0000	0.0000
6	277	3	0.0000	0.0000
6	277	4	1.2500	1.2500
6	277	5	0.0000	0.0000
6	277	6	0.0000	0.0000
6	277	7	0.0000	0.0000
6	277	8	0.0000	0.0000
6	277	9	1.1500	1.1500
6	277	10	0.0000	0.0000
6	277	11	0.0000	0.0000
6	277	12	0.0000	0.0000
6	277	13	0.2000	0.2000
6	277	14	0.2500	0.2500
6	277	15	0.1500	0.1500
6	277	16	0.0000	0.0000
6	277	17	1.0500	1.0500
6	277	18	0.0000	0.0000
6	277	19	0.0000	0.0000
6	277	20	0.0000	0.0000
7	444	1	0.0000	0.0000
7	444	2	0.0000	0.0000
7	444	3	0.0000	0.0000
7	444	4	0.0000	0.0000
7	444	5	0.0000	0.0000
7	444	6	0.0000	0.0000
7	444	7	0.0000	0.0000
7	444	8	0.0000	0.0000
7	444	9	0.0000	0.0000
7	444	10	0.0000	0.0000
7	444	11	0.0000	0.0000

7	444	12	0.0000	0.0000
7	444	13	0.0000	0.0000
7	444	14	0.0000	0.0000
7	444	15	0.0000	0.0000
7	444	16	0.0000	0.0000
7	444	17	0.0000	0.0000
7	444	18	0.0000	0.0000
7	444	19	0.0000	0.0000
7	444	20	0.2500	0.2500

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** Benlate® Fungicide 50 DF; [1-[(butylamino) carbonyl]-1H-benzimidazol-2-yl]-carbamic acid, methyl ester; CAS No. 17804-35-2 for benomyl; Batch No. 1210870300; 50.0% active ingredient; a tan powder.

3. **STUDY TYPE:** 72-3. Estuarine Fish Static Acute Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Graves, W.C. and J.P. Swigert. 1993. Benlate® Fungicide 50 DF: A 96-Hour Static Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). Project No. 112A-110A. Prepared by Wildlife International Ltd., Easton, MD. Submitted by E.I. du Pont de Nemours and Company, Newark, DE. EPA MRID No. 426264-02.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 3/2/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 3/2/93

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

Signature: W. Linkenheimer, 2/07/94
Date: Henry Craven 2/11/94
Brockyon 2 16 94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an estuarine fish acute toxicity study. The 96-hour LC_{50} of 26 mg/l (mean measured concentration of Benlate® 50 DF) classifies Benlate® 50 DF as slightly toxic to sheepshead minnows. The NOEC was 14 mg/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile sheepshead minnows (*Cyprinodon variegatus*) were obtained from in-house cultures. Water temperature during the 14-day period immediately prior to test initiation ranged from 21.0 to 23.0°C. The salinity of the water was 25-27 parts per thousand (ppt) and the pH ranged from 7.6 to 7.8. The fish were fed a commercial flake food and brine shrimp nauplii until 49 hours before the test. The fish were acclimated to the test conditions for approximately 48 hours before the test. No mortality occurred in the population during acclimation.

All fish used in the test were from the same year class. The average length of 10 control organisms at the end of the test was 22 mm (18-24 mm) with an average weight of 0.30 g (0.15-0.42 g).

B. **Test System:** The test chambers were Teflon-lined 25-l polyethylene aquaria filled with 15 l of test solution. The test solution depth was approximately 18 cm. The chambers were indiscriminately positioned in a temperature-controlled environmental chamber (22 ±1°C). The test area was maintained on a 16-hour daylight photoperiod with 30-minute dawn and dusk simulations. The light intensity during the test was approximately 161 lux.

One stock solution was prepared for each treatment replicate using dimethylformamide (DMF) as solvent. The stocks were added to 1500 ml of dilution water, covered, and mixed for two hours before being distributed to the test chambers containing dilution water. The test solutions were mixed with electric mixers for 17 hours prior to test initiation.

Natural seawater, collected at Indian River Inlet, DE, was diluted to the appropriate salinity with well water, aerated, and filtered (25 µm) before use as test dilution water. The salinity of the dilution water was 23-25 ppt and the pH was 7.8-8.0 during the four-week period before the test.

C. **Dosage:** Ninety-six-hour static test. Based on the results of preliminary testing, seven nominal

44
49

concentrations (4.7, 7.8, 13.0, 21.6, 36.0, 60.0, and 100 mg/l), a solvent control, and a dilution water control were used. The concentration of DMF in the solvent control and the highest treatment was 0.5 ml/l.

D. **Design:** Sheepshead minnows were impartially removed in groups of two from holding tanks and distributed to the test chambers until each contained 10 fish. Two replicates were used for the control and each exposure level. The instantaneous loading was 0.20 g/l.

Observations of mortality and treatment-related effects were made at 5.5, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO) and pH were measured in alternating replicates at the beginning of the test and at each 24-hour observation. The temperature of one of the control chambers was monitored continuously for the first 66 hours of the study. Temperature of each replicate vessel was measured at the beginning and end of the test. The salinity of the dilution water control was measured at test initiation.

Water samples from each replicate were collected at test initiation, after 24 hours, and at test termination. The samples were shipped on ice to the sponsor for analysis using high performance liquid chromatography. The concentration of whole test material was calculated from measured concentrations of carbendazim (the main aqueous degradation product of Benlate at neutral pH) and STB (1,3,5-triazino[1,2-a]benzimidazole-2,4(1H,3H)-dione,3-butyl-; an aqueous degradation product formed under basic pH).

E. **Statistics:** The median lethal concentration (LC_{50}) and 95% confidence interval (C.I.) were determined using a computer program developed by Stephan.

12. **REPORTED RESULTS:** Analytical data for water samples collected at 24 and 96 hours were presented in Table 1 (attached). The mean measured concentrations for the test, based on "measured carbendazim" and presented as mg/l of Benlate® 50 DF, were 5.2, 8.5, 14, 24, 36, 47, and 37 mg/l. Undissolved material was observed in the 36, 47, and 37 mg/l test chambers.

No mortality or sublethal effects were noted in the controls and three lowest test concentrations (Table 3, attached). The 37 and 47 mg/l test solutions were turbid which made toxicity observations difficult. By the end of the study,

≥95% mortality had occurred at the three highest test levels.

During the test, DO ranged from 5.0 to 7.3 mg/l (>60% of saturation). The pH values ranged from 7.4 to 7.8. From individual measurements in all test aquaria, the temperature was 21.4-22.9°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC_{50} value for sheepshead minnows was 26 mg/l (95% C.I. = 24-28 mg/l) of formulation. The slope of the concentration response curve was 14. The no mortality and no-observed-effect concentration (NOEC) were 14 mg/l.

The authors explained that flow-through and static-renewal testing was not attempted due to the limited solubility of Benlate® 50 DF and the instability of the active ingredient, Benomyl.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance audits were reported.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

The test material was a formulated product. This study should have included a formulation control containing the highest concentration of inert or carrier ingredients present in the highest test level.

The salinity of the dilution water in the study was approximately 25 ppt. The recommended salinity for sheepshead minnows is 10-17 ppt.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour LC_{50} and 95% confidence interval (C.I.) and obtained similar results (see attached printout). For the reasons discussed below, the two highest test concentrations were excluded from the analysis; however, five concentrations were still analyzed.

C. **Discussion/Results:** The concentration of carbendazim and STB in the test solutions on day 1 and day 4 were provided in du Pont Haskell Laboratory report Table 2

46
57

(attached). Values for each were combined to determine the concentration of Benlate® 50 DF as whole product.

Only samples taken from settled, unmixed test solutions were analyzed. Therefore, the samples collected at test initiation were not analyzed and no comparisons were made between measured concentrations of mixed and unmixed samples.

Because of poor solubility, the two highest test concentrations were extremely turbid. Therefore, data from these concentrations were omitted from the analysis. This is justified because little or no more test substance came into solution at concentrations greater than 36 mg/l, five concentrations were analyzed, measured concentrations "flip-flopped" at the higher levels, and mortality at the two highest concentrations (100%) was comparable to that at 36 mg/l (95%).

This study is scientifically sound and meets the guideline requirements for an estuarine fish acute toxicity study. The 96-hour LC_{50} of 26 mg/l (mean measured concentration of Benlate® 50 DF) classifies Benlate® 50 DF as slightly toxic to sheepshead minnows. The NOEC was 14 mg/l.

D. **Adequacy of the Study:**

(1) Classification: Core for a formulated product.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 02-12-93.

EEB Review dated 5/4/1994 Benomyl

Page _____ is not included in this copy.

Pages 48 through 51 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

RIFICI BENLATE SHEEPSHEAD MINNOW 03-02-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
36	20	19	95	2.002716E-03
24	20	6	30	5.765915
14	20	0	0	9.536742E-05
8.5	20	0	0	9.536742E-05
5.2	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 14 AND 36 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 26.81992

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	6.572957E-02	25.87017	23.29498	29.17172

29.17172

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
18	.2366366	1	.999879

SLOPE = 12.38312
95 PERCENT CONFIDENCE LIMITS = 6.359315 AND 18.40693

LC50 = 26.4806
95 PERCENT CONFIDENCE LIMITS = 23.77047 AND 29.29484

LC10 = 20.91071
95 PERCENT CONFIDENCE LIMITS = 16.03186 AND 23.39604

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52

DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
   Shaughnessey No. 099101.

2. **TEST MATERIAL:** DPX-E965-299 (Carbendazim); 1H-benzimidazol-2-yl-carbamic acid, methyl ester; CAS No. 10605-21-7; Lot No. F00701B; 99.3% active ingredient.

3. **STUDY TYPE:** 72-4. Life-Cycle (21-day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Baer, K.N. 1992. Chronic Toxicity of DPX-E965-299 (Carbendazim, MBC) to *Daphnia magna*. Haskell Laboratory Report No. 599-92. Prepared by Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Co., Newark, DE. Submitted by E.I. du Pont de Nemours and Co., Newark, DE. EPA MRID No. 425294-01.

5. **REVIEWED BY:**

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

   Signature: Louis M Rifici
   Date: 3/2/93

6. **APPROVED BY:**

   Pim Kosalwat, Ph.D.
   Senior Scientist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: P. Kosalwat
   Date: 3/2/93

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

   Signature: W. [illegible], 2/07/94
   Date: H.T. Craven 2/11/94
   [illegible] 2-16-94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a chronic, static-renewal toxicity test using the freshwater invertebrate, *Daphnia magna*. The MATC was >0.0031 mg/l and <0.0066 mg/l (geometric mean MATC = 0.004 mg/l) mean measured concentrations, based on the most sensitive parameter, the number of young produced per adult reproductive day.

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

9. **BACKGROUND:**

20

53

58

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** *Daphnia magna* (<24 hours old) were obtained from in-house cultures. Individual adult daphnids were housed in 250-ml glass beakers containing 200 ml of filtered dilution water (20°C). Neonates used in the test were collected from 21-day old adults.

The adult daphnids were fed a combination of two green algae (*Ankistrodesmus falcatus* and *Selenastrum capricornutum*) at a rate of 75,000 cells/ml of each species three times weekly.

B. **Test System:** The test vessels were 250-ml glass beakers containing 200 ml of test solution (7-cm depth). The beakers were randomly placed in a water bath maintained at 19.6-20.0°C. The photoperiod was 16-hour light/8-hour dark with a light intensity of 107-140 lux. Twenty-five-minute low light (2.1 lux) transitions were used to simulate dawn and dusk.

The dilution water was well water which had flowed through aquaria containing fathead minnows. "The ammonia level was not significantly raised based on acceptable culture health."

A stock solution (0.2 mg/ml) was prepared in dimethylformamide (DMF). The stock was used immediately to prepare the test solutions. "All fresh test solutions were confirmed by analysis prior to daphnid transfer." The test solutions were not aerated.

C. **Dosage:** Twenty-one-day, static-renewal, life-cycle chronic toxicity test. Based on a rangefinding test and a previous definitive test, seven nominal concentrations (0.0015, 0.0030, 0.0060, 0.012, 0.025, 0.050, and 0.10 mg/l), a dilution water control, and a solvent control (0.5 ml DMF/l) were selected for the test.

D. **Design:** Each test concentration and control consisted of seven replicate beakers (numbers 1-7) containing one daphnid each, and three replicate beakers (numbers 8-10) containing five daphnids each. Daphnids were randomly added to the control and test beakers. The solutions were renewed every Monday, Wednesday, and Friday. At renewal, the daphnids were fed the same

54
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algae used during culturing for replicates 1-7 and 250,000 cells/ml for replicates 8-10.

Survival, immobility, and the presence of eggs in the brood pouch were monitored daily. The number of normal and immobile offspring produced were determined at renewal on days 9, 12, 14, 16, 19, and 21. The length of the daphnids was determined at test termination.

The dissolved oxygen concentration (DO) and pH were measured in one replicate of all test concentrations at test initiation, in the new and old solutions at each renewal, and at termination. Temperature of the dilution water control was measured daily with a mercury thermometer. The conductivity, hardness, and alkalinity of a dilution water were measured at test initiation, and weekly thereafter.

Samples were taken from the controls and each test level (except the highest concentration) on days 0, 7, 14, and 21. The concentration of carbendazim was determined using high pressure liquid chromatography.

E. **Statistics:** No statistical differences were observed between dilution water control and solvent control data, therefore the data were pooled prior to subsequent analyses. All data were tested for normality (Shapiro-Wilk's test) and homogeneity of variance (Bartlett's test). Survival was analyzed using the Cochran-Armitage Trend test. The 21-day EC_{50} values were determined using probit analysis. The total young per surviving adult, total immobilized young produced, number of days until first brood release, and daphnid length were analyzed using Dunnett's test or Jonckheere's Trend test.

12. **REPORTED RESULTS:** Measured concentrations of carbendazim in the freshly-prepared and old test solutions were presented in Tables 2 and 3 (attached). The overall mean measured concentrations were 0.0016, 0.0031, 0.0066, 0.012, 0.027, 0.050 and 0.10 mg/l.

Survival data from replicates 1-7 (1 daphnid/replicate) and 8-10 (5 daphnids/replicate) were analyzed separately. The number of adults surviving to day 21 was significantly reduced at 0.10 mg/l for replicates 1-7 and 8-10 (Table 4, attached). The 21-day EC_{50} was 0.069 and 0.066 mg/l, for replicates 1-7 and 8-10, respectively. The number of immobile young produced and the first day of reproduction

55

were significantly different from the pooled control at 0.050 mg/l.

Based on the data from replicates 1-7, the total number of live young produced per surviving adult in 21 days was significantly decreased at 0.0066 mg/l and above (Table 4, attached). However, the decrease in reproduction at 0.0066 mg/l was not considered biologically significant based on the lack of a dose-response relationship from 0.0066 to 0.027 mg/l, the large variability within replicate concentrations, the lack of significant effects in other parameters at these concentrations, and the lack of statistically significant effects in the total number of live young produced per surviving adults in replicates 8-10. Both replicate sets show a statistically and biologically significant decrease in reproduction at 0.050 mg/l.

There was a slight increase in the length of surviving adults at 0.050 mg/l which was not considered biologically significant. Mean lengths in the remaining treatments were similar to pooled control length (Table 4, attached).

The pH of the test solutions ranged from 7.7 to 8.4 and the DO ranged from 8.8 to 9.8 mg/l. The temperature was reported as 19.5-20.3°C during the study. The total alkalinity, hardness, and conductivity of the dilution water were 77-80 mg/l as $CaCO_3$, 75-83 mg/l as $CaCO_3$, and 170-190 µmhos/cm, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 21-day no observed effect concentration (NOEC) was 0.027 mg/l and the maximum acceptable toxicant concentration (MATC) was 0.037 mg/l.

Quality Assurance documentation was provided in the report. A GLP statement was included indicating adherence to USEPA GLP Regulations for FIFRA (40 CFR 160).

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP and ASTM (1988), except for the following:

The dilution water was well water which had passed through a fish culture unit. No justification was provided why a fresh dilution water was not used.

Only the conductivity, hardness, and alkalinity of the dilution water were measured weekly. ASTM states that

56

these parameters must be measured in the control, low, medium, and high concentration test solutions weekly.

The light intensity used during the test (107-140 lux) was lower than that recommended by the SEP (400-800 lux).

Test beakers should be covered to reduce evaporation. The report does not state if the beakers were covered.

Treatments must be randomly assigned to the test chambers. The report does not mention if the treatments were randomly assigned.

Length was measured to the nearest 0.1 mm; the SEP recommends measurement to the nearest 0.01 mm.

B. **Statistical Analysis:** By visual inspection of the survival data (Table 4, attached), the only test concentration with treatment-related reduced survival was 0.10 mg/l mean measured concentration. Statistical analysis of survival was not attempted.

The reviewer calculated the average number of young produced per adult reproductive day from information provided in Table 9 (attached). The first reproductive day, number of young produced per adult reproductive day, and daphnid length data were analyzed using Williams' or Bonferroni's tests (Toxstat Version 3.3). Only data from replicates 7-10 (those replicates containing a single daphnid) were analyzed. All comparisons were made to the solvent control.

The first reproductive day was significantly delayed at the 0.05 mg/l test level (printout 1, attached). No daphnids reproduced at 0.10 mg/l. Daphnid reproductive output was significantly reduced at concentrations ≥0.0066 mg/l (printout 2, attached). Though disputed by the author, the decrease in reproduction loosely followed the concentration gradient and is therefore considered treatment-related. Daphnid length was unaffected by the test material (printout 3, attached).

Based on the reviewer's analysis, the MATC was >0.0031 and <0.0066 mg/l.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a chronic, static-renewal toxicity test using the freshwater invertebrate, *Daphnia magna*. The MATC, based on the

most sensitive parameter, the averaged number of young produced per adult reproductive day, was >0.0031 mg/l and <0.0066 mg/l (geometric mean MATC = 0.004 mg/l) mean measured concentrations.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 02-15-93.

REFERENCES: ASTM. 1988. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. E 1193-87.

58
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EEB Review dated 5/9/1994 Benomyl

Page _____ is not included in this copy.

Pages 59 through 65 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

425294-01, CARBENDAZIM, DAPHNIA 1ST REPRODUCTIVE DAY
File: 42529401.DT1 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
Data PASS normality test. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	7	10.143	10.143	9.786
2	DILUTION CONTRL	7	9.429	9.429	9.786
3	0.0016 MG/L	6	11.000	11.000	10.353
4	0.0031	7	10.000	10.000	10.353
5	0.0066	7	10.286	10.286	10.353
6	0.012	7	10.429	10.429	10.353
7	0.027	7	10.143	10.143	10.353
8	0.05	7	13.571	13.571	13.571

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	9.786				
DILUTION CONTRL	9.786	1.017		1.68	k= 1, v=47
0.0016 MG/L	10.353	0.575		1.76	k= 2, v=47
0.0031	10.353	0.598		1.79	k= 3, v=47
0.0066	10.353	0.598		1.80	k= 4, v=47
0.012	10.353	0.598		1.80	k= 5, v=47
0.027	10.353	0.598		1.81	k= 6, v=47
0.05	13.571	9.763	*	1.81	k= 7, v=47

s = 0.657
Note: df used for table values are approximate when v > 20.

425294-01, CARBENDAZIM, YOUNG/ADULT REPROD. DAY
File: 42529401.DT2 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
Data PASS normality test. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	14.8143	CALCULATED t VALUE =	1.0270
GRP2 (BLANK CRTL) MEAN =	12.7857	DEGREES OF FREEDOM =	12
DIFFERENCE IN MEANS =	2.0286		

TABLE t VALUE (0.05 (2),12) = 2.179 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha=0.01

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	7	14.814	14.814	14.814
2	DILUTION CONTRL	7	12.786	12.786	14.215
3	0.0016 MG/L	6	15.883	15.883	14.215
4	0.0031	7	13.200	13.200	13.200
5	0.0066	7	11.771	11.771	12.052
6	0.012	7	11.929	11.929	12.052
7	0.027	7	12.457	12.457	12.052
8	0.05	7	6.100	6.100	6.100

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	14.814				
DILUTION CONTRL	14.215	0.402		1.68	k= 1, v=47
0.0016 MG/L	14.215	0.386		1.76	k= 2, v=47
0.0031	13.200	1.083		1.79	k= 3, v=47
0.0066	12.052	1.853	*	1.80	k= 4, v=47
0.012	12.052	1.853	*	1.80	k= 5, v=47
0.027	12.052	1.853	*	1.81	k= 6, v=47
0.05	6.100	5.846	*	1.81	k= 7, v=47

s = 2.789
Note: df used for table values are approximate when v > 20.

425294-01, CARBENDAZIM, DAPHNID LENGTH
File: A:42529401.DT3 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
Data PASS normality test. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	3.8857	CALCULATED t VALUE =	-0.3397
GRP2 (BLANK CRTL) MEAN =	3.9143	DEGREES OF FREEDOM =	12
DIFFERENCE IN MEANS =	-0.0286		

TABLE t VALUE (0.05 (2),12) = 2.179 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	0.131	0.019	1.264
Within (Error)	47	0.697	0.015	
Total	54	0.828		

Critical F value = 2.25 (0.05,7,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	SOLVENT CONTROL	3.886	3.886		
2	DILUTION CONTRL	3.914	3.914	-0.439	
3	0.0016 MG/L	3.800	3.800	1.265	
4	0.0031	3.943	3.943	-0.878	
5	0.0066	3.914	3.914	-0.439	
6	0.012	3.914	3.914	-0.439	
7	0.027	3.943	3.943	-0.878	
8	0.05	3.986	3.986	-1.536	

Bonferroni T table value = 2.56 (1 Tailed Value, P=0.05, df=40,7)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	SOLVENT CONTROL	7			
2	DILUTION CONTRL	7	0.167	4.3	-0.029
3	0.0016 MG/L	6	0.174	4.5	0.086
4	0.0031	7	0.167	4.3	-0.057
5	0.0066	7	0.167	4.3	-0.029
6	0.012	7	0.167	4.3	-0.029
7	0.027	7	0.167	4.3	-0.057
8	0.05	7	0.167	4.3	-0.100

TITLE: 425294-01, CARBENDAZIM, DAPHNIA 1ST REPRODUCTIVE DAY
FILE: 42529401.DT1
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	9.0000	9.0000
1	SOLVENT CONTROL	2	11.0000	11.0000
1	SOLVENT CONTROL	3	10.0000	10.0000
1	SOLVENT CONTROL	4	10.0000	10.0000
1	SOLVENT CONTROL	5	11.0000	11.0000
1	SOLVENT CONTROL	6	10.0000	10.0000
1	SOLVENT CONTROL	7	10.0000	10.0000
2	DILUTION CONTRL	1	9.0000	9.0000
2	DILUTION CONTRL	2	9.0000	9.0000
2	DILUTION CONTRL	3	11.0000	11.0000
2	DILUTION CONTRL	4	9.0000	9.0000
2	DILUTION CONTRL	5	10.0000	10.0000
2	DILUTION CONTRL	6	9.0000	9.0000
2	DILUTION CONTRL	7	9.0000	9.0000
3	0.0016 MG/L	1	10.0000	10.0000
3	0.0016 MG/L	2	12.0000	12.0000
3	0.0016 MG/L	3	10.0000	10.0000
3	0.0016 MG/L	4	12.0000	12.0000
3	0.0016 MG/L	5	10.0000	10.0000
3	0.0016 MG/L	6	12.0000	12.0000
4	0.0031	1	10.0000	10.0000
4	0.0031	2	11.0000	11.0000
4	0.0031	3	10.0000	10.0000
4	0.0031	4	10.0000	10.0000
4	0.0031	5	10.0000	10.0000
4	0.0031	6	9.0000	9.0000
4	0.0031	7	10.0000	10.0000
5	0.0066	1	10.0000	10.0000
5	0.0066	2	10.0000	10.0000
5	0.0066	3	11.0000	11.0000
5	0.0066	4	11.0000	11.0000
5	0.0066	5	10.0000	10.0000
5	0.0066	6	10.0000	10.0000
5	0.0066	7	10.0000	10.0000
6	0.012	1	10.0000	10.0000
6	0.012	2	11.0000	11.0000
6	0.012	3	11.0000	11.0000
6	0.012	4	10.0000	10.0000
6	0.012	5	11.0000	11.0000
6	0.012	6	10.0000	10.0000
6	0.012	7	10.0000	10.0000
7	0.027	1	10.0000	10.0000
7	0.027	2	10.0000	10.0000
7	0.027	3	10.0000	10.0000
7	0.027	4	10.0000	10.0000
7	0.027	5	11.0000	11.0000
7	0.027	6	10.0000	10.0000
7	0.027	7	10.0000	10.0000
8	0.05	1	14.0000	14.0000
8	0.05	2	13.0000	13.0000
8	0.05	3	13.0000	13.0000
8	0.05	4	13.0000	13.0000
8	0.05	5	14.0000	14.0000
8	0.05	6	14.0000	14.0000
8	0.05	7	14.0000	14.0000

TITLE: 425294-01, CARBENDAZIM, YOUNG/ADULT REPROD. DAY
FILE: 42529401.DT2
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	14.3000	14.3000
1	SOLVENT CONTROL	2	21.6000	21.6000
1	SOLVENT CONTROL	3	13.4000	13.4000
1	SOLVENT CONTROL	4	8.4000	8.4000
1	SOLVENT CONTROL	5	17.5000	17.5000
1	SOLVENT CONTROL	6	18.3000	18.3000
1	SOLVENT CONTROL	7	10.2000	10.2000
2	DILUTION CONTRL	1	14.8000	14.8000
2	DILUTION CONTRL	2	11.0000	11.0000
2	DILUTION CONTRL	3	11.6000	11.6000
2	DILUTION CONTRL	4	11.6000	11.6000
2	DILUTION CONTRL	5	14.9000	14.9000
2	DILUTION CONTRL	6	9.7000	9.7000
2	DILUTION CONTRL	7	15.9000	15.9000
3	0.0016 MG/L	1	14.8000	14.8000
3	0.0016 MG/L	2	14.0000	14.0000
3	0.0016 MG/L	3	18.6000	18.6000
3	0.0016 MG/L	4	16.9000	16.9000
3	0.0016 MG/L	5	13.2000	13.2000
3	0.0016 MG/L	6	17.8000	17.8000
4	0.0031	1	15.6000	15.6000
4	0.0031	2	14.4000	14.4000
4	0.0031	3	10.1000	10.1000
4	0.0031	4	12.5000	12.5000
4	0.0031	5	12.5000	12.5000
4	0.0031	6	10.7000	10.7000
4	0.0031	7	16.6000	16.6000
5	0.0066	1	8.5000	8.5000
5	0.0066	2	11.7000	11.7000
5	0.0066	3	17.6000	17.6000
5	0.0066	4	10.9000	10.9000
5	0.0066	5	12.4000	12.4000
5	0.0066	6	8.6000	8.6000
5	0.0066	7	12.7000	12.7000
6	0.012	1	11.1000	11.1000
6	0.012	2	10.2000	10.2000
6	0.012	3	15.3000	15.3000
6	0.012	4	12.5000	12.5000
6	0.012	5	11.3000	11.3000
6	0.012	6	10.9000	10.9000
6	0.012	7	12.2000	12.2000
7	0.027	1	10.8000	10.8000
7	0.027	2	10.8000	10.8000
7	0.027	3	13.5000	13.5000
7	0.027	4	11.5000	11.5000
7	0.027	5	15.0000	15.0000
7	0.027	6	10.9000	10.9000
7	0.027	7	14.7000	14.7000
8	0.05	1	2.6000	2.6000
8	0.05	2	2.6000	2.6000
8	0.05	3	9.9000	9.9000
8	0.05	4	6.0000	6.0000
8	0.05	5	6.6000	6.6000
8	0.05	6	8.9000	8.9000
8	0.05	7	6.1000	6.1000

70
75

TITLE: 425294-01, CARBENDAZIM, DAPHNID LENGTH
FILE: A:42529401.DT3
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	3.8000	3.8000
1	SOLVENT CONTROL	2	3.8000	3.8000
1	SOLVENT CONTROL	3	3.9000	3.9000
1	SOLVENT CONTROL	4	4.0000	4.0000
1	SOLVENT CONTROL	5	3.9000	3.9000
1	SOLVENT CONTROL	6	4.0000	4.0000
1	SOLVENT CONTROL	7	3.8000	3.8000
2	DILUTION CONTRL	1	4.1000	4.1000
2	DILUTION CONTRL	2	4.0000	4.0000
2	DILUTION CONTRL	3	3.6000	3.6000
2	DILUTION CONTRL	4	3.8000	3.8000
2	DILUTION CONTRL	5	3.9000	3.9000
2	DILUTION CONTRL	6	4.2000	4.2000
2	DILUTION CONTRL	7	3.8000	3.8000
3	0.0016 MG/L	1	3.8000	3.8000
3	0.0016 MG/L	2	3.9000	3.9000
3	0.0016 MG/L	3	4.0000	4.0000
3	0.0016 MG/L	4	3.8000	3.8000
3	0.0016 MG/L	5	3.7000	3.7000
3	0.0016 MG/L	6	3.6000	3.6000
4	0.0031	1	4.0000	4.0000
4	0.0031	2	4.1000	4.1000
4	0.0031	3	3.8000	3.8000
4	0.0031	4	4.0000	4.0000
4	0.0031	5	4.0000	4.0000
4	0.0031	6	3.9000	3.9000
4	0.0031	7	3.8000	3.8000
5	0.0066	1	3.8000	3.8000
5	0.0066	2	3.8000	3.8000
5	0.0066	3	3.9000	3.9000
5	0.0066	4	3.9000	3.9000
5	0.0066	5	4.1000	4.1000
5	0.0066	6	4.0000	4.0000
5	0.0066	7	3.9000	3.9000
6	0.012	1	4.0000	4.0000
6	0.012	2	3.9000	3.9000
6	0.012	3	3.9000	3.9000
6	0.012	4	4.0000	4.0000
6	0.012	5	3.9000	3.9000
6	0.012	6	3.9000	3.9000
6	0.012	7	3.8000	3.8000
7	0.027	1	3.9000	3.9000
7	0.027	2	3.8000	3.8000
7	0.027	3	4.0000	4.0000
7	0.027	4	3.9000	3.9000
7	0.027	5	4.1000	4.1000
7	0.027	6	3.9000	3.9000
7	0.027	7	4.0000	4.0000
8	0.05	1	4.0000	4.0000
8	0.05	2	3.8000	3.8000
8	0.05	3	4.1000	4.1000
8	0.05	4	4.1000	4.1000
8	0.05	5	4.0000	4.0000
8	0.05	6	3.9000	3.9000
8	0.05	7	4.0000	4.0000

DP Barcode : D191257
PC Code No : 099101
EEB Out APR 5 1994

To: Linda Propst, PM 73
Special Review and Reregisration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 099101
Chemical Name : Benomyl
Type Product : Fungicide
Product Name :
Company Name : DuPont
Purpose : Reregistration data

Action Code : 627 Date Due : 08/11/93
Reviewer : William A. Erickson

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.
71-1(a)			72-2(a)			72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)			122-1(a)		
71-2(b)			72-3(b)			122-1(b)		
71-3			72-3(c)			122-2		
71-4(a)			72-3(d)			123-1(a)		
71-4(b)			72-3(e)			123-1(b)		
71-5(a)			72-3(f)			123-2		
71-5(b)			72-4(a)			124-1		
72-1(a)			72-4(b)	427237-01	Y	124-2		
72-1(b)			72-5			141-1		
72-1(c)			72-6			141-2		
72-1(d)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

12
77

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

APR 5 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl: review of mysid life-cycle toxicity test

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Linda Propst, PM 73
Special Review and Reregistration Division (7508W)

E.I. du Pont de Nemours and Company, Newark, DE submitted the following study to support reregistration of benomyl:

Ward, T.J. and R.L. Boeri. 1992. Life cycle toxicity of DPX-E965-299 (carbendazim, MBC) to the mysid,(*Mysidopsis bahia*). Conducted by T.R. Wilbury Laboratories, Inc., Marblehead, MA. MRID No. 427237-01.

This study is scientifically sound and fulfills the guideline requirement (72-4b) for a mysid life-cycle toxicity test. Refer to the enclosed Data Evaluation Record for the results and classification of the study.

If you have any questions, please contact Bill Erickson at 305-6212 or Henry Craven at 305-5320.

13

98

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DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl. Shaughnessey No. 099101.

2. **TEST MATERIAL:** DPX-E965-299 (Carbamic acid; 1H-benzimidazol-2-yl-methyl ester); CAS No. 10605-21-7; Lot No. F00701B; 99.3% active ingredient; a tan powder.

3. **STUDY TYPE:** Mysid Life-Cycle Toxicity Test. Species Tested: *Mysidopsis bahia*.

4. **CITATION:** Ward, T.J. and R.L. Boeri. 1992. Life Cycle Toxicity of DPX-E965-299 (Carbendazim, MBC) to the Mysid, *Mysidopsis bahia*. T.R. Wilbury Study No. 97-DU. Prepared by T.R. Wilbury Laboratories, Inc., Marblehead, MA. Submitted by E.I. du Pont de Nemours and Company, Inc., Newark, NJ. EPA MRID No. 427237-01.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 14 June 93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 6/14/93

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

Signature: W. Craven 3/29/94
Date: Henry T. Craven 6/14/94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a mysid life-cycle toxicity test. Based on mean measured concentrations, the MATC of DPX-E965-299 to *Mysidopsis bahia*, was >24.8 and <50.4 µg ai/l (geometric mean = 35.4 µg ai/l).

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

74

11. MATERIALS AND METHODS:

A. Test Animals: Juvenile *Mysidopsis bahia* (<24 hours old) were obtained from in-house cultures. The mysids were free from disease and abnormalities at the beginning of the test. During the ten days prior to test initiation, the acclimation temperature was 24.7-25.5°C.

B. Test System: An intermittent-flow proportional diluter delivered test solutions to 20-l glass aquaria (20 x 40 x 25 cm). The test vessels were equipped with self-starting siphons to ensure adequate flow of test solution to the mysids. Each aquarium contained up to 8 l of test solution (water depth ranged from 3 to 9 cm). The volume of each aquarium was replaced an average of 6.4 times every 24 hours.

From day 0 to 13, two retention chambers (9-cm diameter glass petri dishes with 12-cm high Nitex screen collars) were placed in each of two test vessels per treatment. From day 14 on, twelve retention chambers (6-cm diameter glass petri dishes with 12-cm high Nitex screen collars) were placed in each replicate aquarium.

The target test temperature was 25 ±1°C. A 16-hour light photoperiod with a 15-minute transition period between dark and light was provided daily. The light intensity was 39 footcandles. No aeration was required.

The dilution water (acclimation water) was seawater collected from the Atlantic Ocean in Marblehead, MA. The salinity was adjusted to 16-17 parts per thousand (ppt) using an undescribed method and the pH was 7.7-8.2. The dilution water was stored in 500-gallon polyethylene tanks, aerated, filtered, and uv-sterilized prior to use.

A primary stock solution (2,000 mg/l) was prepared periodically by dissolving appropriate amounts of the test substance in dimethylformamide (DMF).

C. Dosage: Twenty-eight-day flow-through test. Five nominal concentrations (6, 12, 25, 50, and 100 µg/l) were selected for this study. In addition, a dilution water control and a solvent control (0.05 ml DMF/l) were included. The DMF concentration in the solvent control was the same as that present in the highest test concentration.

75
80

D. Design: Sixty mysids were indiscriminately and equally distributed to two replicate aquaria per treatment. Within each aquarium, the thirty mysids were evenly subdivided into two retention chambers. The test aquaria were randomly arranged in a water bath. On day 13 of the exposure, the F_1 mysids were categorized by sex and separated into pairs (1 male and 1 female). Each of ten pairs per replicate were placed into individual retention chambers. Unpaired mysids were placed into two additional retention chambers per replicate. The mysids were fed newly hatched *Artemia salina* nauplii at least twice daily, except for the last day of the test. Test vessels were cleaned daily beginning on day 5.

Observations of mortality and sublethal responses were recorded every 24 hours. Every 1-3 days, the offspring produced in each cage were counted and removed. Dead F_1 mysids were removed when first observed. The total length and wet weight of individual mysids were determined at test termination. Mean dry weight (60°C, 72 hours) was also determined.

Temperature, dissolved oxygen concentration (DO), salinity, and pH were measured daily in each aquarium. In addition, the temperature in one aquarium was recorded continuously during the study.

Concentrations of the test material in samples collected from each test vessel on days 0, 7, 14, 21, and 28 were analyzed using high performance liquid chromatography.

E. Statistics: Six biological measures of effect were statistically analyzed: (1) number of F_1 mysids surviving the exposure period, (2) number of young per surviving female after 28 days of exposure, (3) number of young produced per reproductive day, (4) total length of surviving F_1 mysids, (5) wet weight of surviving F_1 mysids, and (6) dry weight of surviving F_1 mysids. Control and solvent control data were not significantly different (t-test), except for wet weight data. Therefore, treatment responses were compared with those of the pooled controls (wet weight data were analyzed relative to each the control and solvent control data). Bartlett's test was used to determine whether variances were homogeneous and Shapiro-Wilk's test was used to determine whether the data were normally distributed. Homogeneous data were analyzed

76

using a one-way analysis of variance (ANOVA), and if necessary, Fisher's Exact test for survival data or Bonferroni's test to compare control and treatment means. If the data were heteroscedastic, a non-parametric ANOVA was used. Statistical significance was concluded at the 95% confidence level.

12. **REPORTED RESULTS:** No insoluble material was observed in any test vessel during the study. Mean measured concentrations were 5.61, 11.6, 24.8, 50.4, and 100 µg ai/l (Table 1, attached).

Mysid survival, wet weight, and dry weight at 100 µg ai/l were significantly lower than the control or pooled control data (Table 3, attached). Reproductive success at concentrations ≥50.4 µg ai/l was significantly lower (or assumed to be significantly lower) than that of the pooled control. Total length in the exposure solutions was not significantly lower than that of the pooled control. No sublethal effects were observed at concentrations below 50.4 µg ai/l. After 28 days, the LOEC and NOEC were 50.4 and 24.8 µg ai/l, respectively (Table 5, attached). The maximum acceptable toxicant concentration (MATC) was 35.4 µg ai/l.

During the study, the test solutions had a pH of 7.7-8.3, a temperature of 24.1-25.1°C, a salinity of 16-17 ppt, and a DO of 5.6-7.9 mg/l.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors presented no conclusions.

Good laboratory practice and quality assurance statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. **Test Procedure:** ASTM guidelines (1990) were used to evaluate this study. The test procedures generally followed the recommended protocols. The deviations are noted as follows:

The dry weight of individual surviving mysids must be determined at the end of the exposure period. Only mean dry weights were determined in this study.

The temperature during the test (24.1-25.1°C) was lower than recommended (27°C).

77
82

B. **Statistical Analysis:** For each parameter analyzed, the responses of the treatments were compared to the dilution water control since the solvent was not present at the same level in all test solutions. The reviewer used computer programs (Toxstat 3.3 or SYSTAT®) to analyze the number of adult mysids surviving the exposure period, the total number of young produced per average number of surviving females, the number of young produced per female reproductive day (as calculated by the authors and the reviewer), the length of surviving mysids, and the wet and dry weights of surviving mysids. Wet weights and lengths of male and female mysids were analyzed separately.

The reviewer determined the number of young produced per female reproductive day by dividing the total number of young per replicate by the number of female reproductive days for that replicate (i.e., the number of days beginning on the first day young were observed in that replicate). The reviewer analyzed the reproduction data presented by the authors and the number of young per reproductive day as determined by the reviewer.

Male dry weight, reproduction, and survival data did not meet the assumptions of homogeneity of variance, therefore, these data were analyzed using the Kruskal-Wallis test. No significant difference between the dilution water control and the exposure solutions were determined. However, upon visual examination of the data, there was a substantial reduction in survival and reproduction at 50.4 and 100 µg ai/l (pages 5, 10, 15, and 20 of printouts, attached). Male dry weights were substantially reduced at 100 µg ai/l (page 25 of printouts, attached). Female dry weight data were analyzed using William's test which demonstrated a significant reduction at 100 µg ai/l (page 29 of printouts, attached). The total length and wet weight data were analyzed using two-way ANOVA and Bonferroni's test (pages 35-38 of printouts, attached). Length and wet weight data for all mysids in the study were provided in the report, but the reviewer only included mature mysids in the statistical analyses. Only female wet weight at 100 µg ai/l was significantly reduced when compared to dilution water control (page 36 of printouts, attached). Some of these conclusions are less conservative than those presented by the authors (Table 5, attached).

C. **Discussion/Results:** This study is scientifically sound

and fulfills the guideline requirements for a mysid life-cycle toxicity test. Based on mean measured concentrations, the MATC value of DPX-E965-299 to the mysid was ≤50.4 and ≥24.8 µg ai/l (the geometric mean MATC = 35.4 µg ai/l).

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes; 27 May 1993.

REFERENCE:

ASTM. 1990. Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids. ASTM Designation: E 1191-90.

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84

EEB Review dated 5/9/1994 Benomyl

Page _____ is not included in this copy.

Pages 80 through 83 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Carbendazim: Survival of Exposed Mysidopsis bahia
File: 42723701.sur Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	6	2	6	0

Calculated Chi-Square goodness of fit test statistic = 7.9994
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Carbendazim: Survival of Exposed Mysidopsis bahia
File: 42723701.sur Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.038

W = 0.942

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

Carbendazim: Survival of Exposed Mysidopsis bahia
File: 42723701.sur Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: Carbendazim: Survival of Exposed Mysidopsis bahia
FILE: 42723701.sur
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.9700	1.3967
1	Control	2	0.9000	1.2490
2	Solvent Control	1	0.8700	1.2019
2	Solvent Control	2	0.9000	1.2490
3	5.61 ug/l	1	0.8700	1.2019
3	5.61 ug/l	2	0.9000	1.2490
4	11.6 ug/l	1	0.9300	1.3030
4	11.6 ug/l	2	0.8300	1.1458
5	24.8 ug/l	1	0.8300	1.1458
5	24.8 ug/l	2	0.8300	1.1458
6	50.4 ug/l	1	0.7300	1.0244
6	50.4 ug/l	2	0.8000	1.1071
7	100 ug/l	1	0.5000	0.7854
7	100 ug/l	2	0.3700	0.6539

87
42

Carbendazim: Survival of Exposed Mysidopsis bahia
File: 42723701.sur Transform: ARC SINE(SQUARE ROOT(Y))

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	1.323	0.935	25.000
2	Solvent Control	1.225	0.885	19.500
3	5.61 ug/l	1.225	0.885	19.500
4	11.6 ug/l	1.224	0.880	19.000
5	24.8 ug/l	1.146	0.830	12.000
6	50.4 ug/l	1.066	0.765	7.000
7	100 ug/l	0.720	0.435	3.000

Calculated H Value = 10.887 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Carbendazim: Survival of Exposed Mysidopsis bahia
File: 42723701.sur Transform: ARC SINE(SQUARE ROOT(Y))

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 7	0 6	0 5	0 4	0 2	0 3	0 1
7	100 ug/l	0.720	0.435	\						
6	50.4 ug/l	1.066	0.765	.	\					
5	24.8 ug/l	1.146	0.830	.	.	\				
4	11.6 ug/l	1.224	0.880	.	.	.	\			
2	Solvent Control	1.225	0.885	.	.	.	.	\		
3	5.61 ug/l	1.225	0.885	.	.	.	.	.	\	
1	Control	1.323	0.935	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.142

Carbendazim: N. Young per Female Reproductive Day (Reviewer's Calc)
File: 42723701.rev Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	6	2	6	0

Calculated Chi-Square goodness of fit test statistic = 7.9994
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

89
84

Carbendazim: N. Young per Female Reproductive Day (Reviewer's Calc.)
File: 42723701.rev Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.068

W = 0.967

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

Carbendazim: N. Young per Female Reproductive Day (Reviewer Calc)
File: 42723701.rev Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

91

46

TITLE: Carbendazim: N. Young per Female Reproductive Day (Reviewer's Calc)
FILE: 42723701.rev
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.8900	0.8900
1	Control	2	0.7600	0.7600
2	Solvent Control	1	0.9500	0.9500
2	Solvent Control	2	0.7500	0.7500
3	5.61 ug/l	1	1.0400	1.0400
3	5.61 ug/l	2	0.8100	0.8100
4	11.6 ug/l	1	0.8400	0.8400
4	11.6 ug/l	2	0.9800	0.9800
5	24.8 ug/l	1	0.7600	0.7600
5	24.8 ug/l	2	0.8400	0.8400
6	50.4 ug/l	1	0.0200	0.0200
6	50.4 ug/l	2	0.0500	0.0500
7	100 ug/l	1	0.0000	0.0000
7	100 ug/l	2	0.0000	0.0000

92

Carbendazim: N. Young per Female Reproductive Day (Reviewers Calc).
File: 42723701.rev Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	0.825	0.825	17.500
2	Solvent Control	0.850	0.850	17.000
3	5.61 ug/l	0.925	0.925	22.000
4	11.6 ug/l	0.910	0.910	22.500
5	24.8 ug/l	0.800	0.800	16.000
6	50.4 ug/l	0.035	0.035	7.000
7	100 ug/l	0.000	0.000	3.000

Calculated H Value = 9.333 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Carbendazim: N. Young per Female Reproductive Day
File: 42723701.rev Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 7	0 6	0 5	0 1	0 2	0 4	0 3
7	100 ug/l	0.000	0.000	\						
6	50.4 ug/l	0.035	0.035	.	\					
5	24.8 ug/l	0.800	0.800	.	.	\				
1	Control	0.825	0.825	.	.	.	\			
2	Solvent Control	0.850	0.850	.	.	.	.	\		
4	11.6 ug/l	0.910	0.910	.	.	.	.	.	\	
3	5.61 ug/l	0.925	0.925	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.169

93

98

Carbendazim: Total No. Young/Average No. Females
File: 42723701.avg Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	6	2	6	0

Calculated Chi-Square goodness of fit test statistic = 7.9994
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

94
99

Carbendazim: Total No. Young/Average No. Females
File: 42723701.avg Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 11.215

W = 0.964

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

95
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Carbendazim: Total No. Young/Average No. Females
File: 42723701.avg Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

96

TITLE: Carbendazim: Total No. Young/Average No. Females
FILE: 42723701.avg
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	11.6000	11.6000
1	Control	2	9.9000	9.9000
2	Solvent Control	1	11.4000	11.4000
2	Solvent Control	2	9.8000	9.8000
3	5.61 ug/l	1	13.5000	13.5000
3	5.61 ug/l	2	10.5000	10.5000
4	11.6 ug/l	1	10.1000	10.1000
4	11.6 ug/l	2	12.7000	12.7000
5	24.8 ug/l	1	9.8000	9.8000
5	24.8 ug/l	2	10.9000	10.9000
6	50.4 ug/l	1	0.2000	0.2000
6	50.4 ug/l	2	0.1000	0.1000
7	100 ug/l	1	0.0000	0.0000
7	100 ug/l	2	0.0000	0.0000

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Carbendazim: Total No. Young/Average No. Females
File: 42723701.avg Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	10.750	10.750	19.000
2	Solvent Control	10.600	10.600	16.500
3	5.61 ug/l	12.000	12.000	23.000
4	11.6 ug/l	11.400	11.400	21.000
5	24.8 ug/l	10.350	10.350	15.500
6	50.4 ug/l	0.150	0.150	7.000
7	100 ug/l	0.000	0.000	3.000

Calculated H Value = 9.370 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Carbendazim: Total No. Young/Average No. Females
File: 42723701.avg Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 7 6 5 2 1 4 3
7	100 ug/l	0.000	0.000	\
6	50.4 ug/l	0.150	0.150	. \
5	24.8 ug/l	10.350	10.350	. . \
2	Solvent Control	10.600	10.600	. . . \
1	Control	10.750	10.750	. . . . \
4	11.6 ug/l	11.400	11.400	. . . . . \
3	5.61 ug/l	12.000	12.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.174

98

Carbendazim: N. Young per Female Reproductive Day (Authors' Calc.)
File: 42723701.rdy Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	6	2	6	0

Calculated Chi-Square goodness of fit test statistic = 7.9994
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Carbendazim: N. Young per Female Reproductive Day (Authors' Calc)
File: 42723701.rdy Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.067

W = 0.960

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

Carbendazim: N. Young per Female Reproductive Day (Authors' Calc)
File: 42723701.rdy Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: Carbendazim: N. Young per Female Reproductive Day (Authors' Calc)
FILE: 42723701.rdy
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.8900	0.8900
1	Control	2	0.7600	0.7600
2	Solvent Control	1	0.8800	0.8800
2	Solvent Control	2	0.7500	0.7500
3	5.61 ug/l	1	1.0400	1.0400
3	5.61 ug/l	2	0.8100	0.8100
4	11.6 ug/l	1	0.7800	0.7800
4	11.6 ug/l	2	0.9800	0.9800
5	24.8 ug/l	1	0.7500	0.7500
5	24.8 ug/l	2	0.8400	0.8400
6	50.4 ug/l	1	0.0200	0.0200
6	50.4 ug/l	2	0.0100	0.0100
7	100 ug/l	1	0.0000	0.0000
7	100 ug/l	2	0.0000	0.0000

102

Carbendazim: N. Young per Female Reproductive Day (Author inc
File: 42723701.rdy Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	0.825	0.825	19.000
2	Solvent Control	0.815	0.815	16.500
3	5.61 ug/l	0.925	0.925	23.000
4	11.6 ug/l	0.880	0.880	21.000
5	24.8 ug/l	0.795	0.795	15.500
6	50.4 ug/l	0.015	0.015	7.000
7	100 ug/l	0.000	0.000	3.000

Calculated H Value = 9.370 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Carbendazim: N. Young per Female Reproductive Day
File: 42723701.rdy Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 7	0 6	0 5	0 2	0 1	0 4	0 3
7	100 ug/l	0.000	0.000	\						
6	50.4 ug/l	0.015	0.015	.	\					
5	24.8 ug/l	0.795	0.795	.	.	\				
2	Solvent Control	0.815	0.815	.	.	.	\			
1	Control	0.825	0.825	.	.	.	.	\		
4	11.6 ug/l	0.880	0.880	.	.	.	.	.	\	
3	5.61 ug/l	0.925	0.925	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.174

Carbendazim: Dry Weight of Exposed Male Mysids
File: 42723701.mdw Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	6	2	6	0

Calculated Chi-Square goodness of fit test statistic = 7.9994
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Carbendazim: Dry Weight of Exposed Male Mysids
File: 42723701.mdw Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.011

W = 0.985

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

Carbendazim: Dry Weight of Exposed Male Mysids
File: 42723701.mdw Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

166
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TITLE: Carbendazim: Dry Weight of Exposed Male Mysids
FILE: 42723701.mdw
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.6700	0.6700
1	Control	2	0.6200	0.6200
2	Solvent Control	1	0.6400	0.6400
2	Solvent Control	2	0.6200	0.6200
3	5.61 ug/l	1	0.6800	0.6800
3	5.61 ug/l	2	0.6700	0.6700
4	11.6 ug/l	1	0.5900	0.5900
4	11.6 ug/l	2	0.6400	0.6400
5	24.8 ug/l	1	0.7000	0.7000
5	24.8 ug/l	2	0.6400	0.6400
6	50.4 ug/l	1	0.6500	0.6500
6	50.4 ug/l	2	0.6500	0.6500
7	100 ug/l	1	0.4300	0.4300
7	100 ug/l	2	0.3200	0.3200

Carbendazim: Dry Weight of Exposed Male Mysids
File: 42723701.mdw Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	0.645	0.645	16.000
2	Solvent Control	0.630	0.630	11.500
3	5.61 ug/l	0.675	0.675	24.500
4	11.6 ug/l	0.615	0.615	10.000
5	24.8 ug/l	0.670	0.670	21.000
6	50.4 ug/l	0.650	0.650	19.000
7	100 ug/l	0.375	0.375	3.000

Calculated H Value = 9.416 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Carbendazim: Dry Weight of Exposed Male Mysids
File: 42723701.mdw Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 7	0 4	0 2	0 1	0 6	0 5	0 3
7	100 ug/l	0.375	0.375	\						
4	11.6 ug/l	0.615	0.615	.	\					
2	Solvent Control	0.630	0.630	.	.	\				
1	Control	0.645	0.645	.	.	.	\			
6	50.4 ug/l	0.650	0.650	.	.	.	.	\		
5	24.8 ug/l	0.670	0.670	.	.	.	.	.	\	
3	5.61 ug/l	0.675	0.675	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.151

Carbendazim: Dry Weight of Exposed Female Mysids
File: 42723701.fdw Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.045

W = 0.979

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

Carbendazim: Dry Weight of Exposed Female Mysids
File: 42723701.fdw Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 3.86
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: Carbendazim: Dry Weight of Exposed Female Mysids
FILE: 42723701.fdw
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.8900	0.8900
1	Control	2	0.8300	0.8300
2	Solvent Control	1	0.8200	0.8200
2	Solvent Control	2	0.6800	0.6800
3	5.61 ug/l	1	0.9500	0.9500
3	5.61 ug/l	2	0.8800	0.8800
4	11.6 ug/l	1	0.9000	0.9000
4	11.6 ug/l	2	0.9800	0.9800
5	24.8 ug/l	1	0.8100	0.8100
5	24.8 ug/l	2	0.8300	0.8300
6	50.4 ug/l	1	0.6900	0.6900
6	50.4 ug/l	2	0.7700	0.7700
7	100 ug/l	1	0.6400	0.6400
7	100 ug/l	2	0.4200	0.4200

111
46

Carbendazim: Dry Weight of Exposed Female Mysids
File: 42723701.fdw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	2	0.860	0.860	0.866
2	Solvent Control	2	0.750	0.750	0.866
3	5.61 ug/l	2	0.915	0.915	0.866
4	11.6 ug/l	2	0.940	0.940	0.866
5	24.8 ug/l	2	0.820	0.820	0.820
6	50.4 ug/l	2	0.730	0.730	0.730
7	100 ug/l	2	0.530	0.530	0.530

Carbendazim: Dry Weight of Exposed Female Mysids
File: 42723701.fdw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.866				
Solvent Control	0.866	0.078		1.89	k= 1, v= 7
5.61 ug/l	0.866	0.078		2.00	k= 2, v= 7
11.6 ug/l	0.866	0.078		2.04	k= 3, v= 7
24.8 ug/l	0.820	0.500		2.06	k= 4, v= 7
50.4 ug/l	0.730	1.624		2.07	k= 5, v= 7
100 ug/l	0.530	4.122	*	2.08	k= 6, v= 7

s = 0.080
Note: df used for table values are approximate when v > 20.

112

Carbendazim : Mysidopsis bahia

TRT 1 = DILUTION WATER CONTROL
TRT 2 = Solvent Control
TRT 3 = 5.61 ug/l
TRT 4 = 11.6 ug/l
TRT 5 = 24.8 ug/l
TRT 6 = 50.4 ug/l
TRT 7 = 100 ug/l

SEX 1=MALE
SEX 2=FEMALE

		TRT	REP	SEX	WEIGHT	LENGTH
CASE	1	1.0000	1.0000	1.0000	0.0007	7.0000
CASE	2	1.0000	1.0000	1.0000	0.0009	6.6000
CASE	3	1.0000	1.0000	1.0000	0.0007	7.4000
CASE	4	1.0000	1.0000	1.0000	0.0007	7.3000
CASE	5	1.0000	1.0000	1.0000	0.0006	7.8000
CASE	6	1.0000	1.0000	1.0000	0.0006	7.0000
CASE	7	1.0000	1.0000	1.0000	0.0007	7.4000
CASE	8	1.0000	1.0000	1.0000	0.0007	8.0000
CASE	9	1.0000	1.0000	1.0000	0.0005	6.8000
CASE	10	1.0000	1.0000	1.0000	0.0008	6.2000
CASE	11	1.0000	1.0000	1.0000	0.0007	7.5000
CASE	12	1.0000	1.0000	1.0000	0.0008	7.7000
CASE	13	1.0000	1.0000	1.0000	0.0006	6.2000
CASE	14	1.0000	1.0000	1.0000	0.0007	7.5000
CASE	15	1.0000	1.0000	2.0000	0.0012	7.5000
CASE	16	1.0000	1.0000	2.0000	0.0011	6.6000
CASE	17	1.0000	1.0000	2.0000	0.0009	7.5000
CASE	18	1.0000	1.0000	2.0000	0.0011	7.1000
CASE	19	1.0000	1.0000	2.0000	0.0007	7.6000
CASE	20	1.0000	1.0000	2.0000	0.0008	8.4000
CASE	21	1.0000	1.0000	2.0000	0.0007	7.2000
CASE	22	1.0000	1.0000	2.0000	0.0006	7.2000
CASE	23	1.0000	1.0000	2.0000	0.0010	7.7000
CASE	24	1.0000	1.0000	2.0000	0.0007	7.6000
CASE	25	1.0000	1.0000	2.0000	0.0010	7.0000
CASE	26	1.0000	1.0000	2.0000	0.0011	7.6000
CASE	27	1.0000	1.0000	2.0000	0.0012	7.6000
CASE	28	1.0000	1.0000	2.0000	0.0011	7.6000
CASE	29	1.0000	1.0000	2.0000	0.0011	7.8000
CASE	30	1.0000	2.0000	1.0000	0.0006	6.2000
CASE	31	1.0000	2.0000	1.0000	0.0008	6.5000
CASE	32	1.0000	2.0000	1.0000	0.0006	6.8000
CASE	33	1.0000	2.0000	1.0000	0.0006	6.8000
CASE	34	1.0000	2.0000	1.0000	0.0006	7.1000
CASE	35	1.0000	2.0000	1.0000	0.0009	7.5000
CASE	36	1.0000	2.0000	1.0000	0.0006	7.2000
CASE	37	1.0000	2.0000	1.0000	0.0007	8.0000
CASE	38	1.0000	2.0000	1.0000	0.0005	8.4000
CASE	39	1.0000	2.0000	1.0000	0.0005	7.2000
CASE	40	1.0000	2.0000	1.0000	0.0007	7.5000
CASE	41	1.0000	2.0000	1.0000	0.0004	6.8000
CASE	42	1.0000	2.0000	1.0000	0.0007	7.0000
CASE	43	1.0000	2.0000	1.0000	0.0008	7.0000
CASE	44	1.0000	2.0000	1.0000	0.0007	7.4000
CASE	45	1.0000	2.0000	1.0000	0.0007	8.0000
CASE	46	1.0000	2.0000	2.0000	0.0013	7.8000
CASE	47	1.0000	2.0000	2.0000	0.0009	7.7000
CASE	48	1.0000	2.0000	2.0000	0.0011	8.1000
CASE	49	1.0000	2.0000	2.0000	0.0008	9.2000
CASE	50	1.0000	2.0000	2.0000	0.0009	7.0000
CASE	51	1.0000	2.0000	2.0000	0.0006	7.5000
CASE	52	1.0000	2.0000	2.0000	0.0007	8.6000
CASE	53	1.0000	2.0000	2.0000	0.0011	7.4000
CASE	54	1.0000	2.0000	2.0000	0.0008	8.4000
CASE	55	1.0000	2.0000	2.0000	0.0009	8.4800
CASE	56	1.0000	2.0000	2.0000	0.0010	8.4000
CASE	57	2.0000	1.0000	1.0000	0.0006	7.5000

CASE	58	2.0000	1.0000	1.0000	0.0006	7.3000
CASE	59	2.0000	1.0000	1.0000	0.0006	7.4000
CASE	60	2.0000	1.0000	1.0000	0.0006	7.6000
CASE	61	2.0000	1.0000	1.0000	0.0008	8.0000
CASE	62	2.0000	1.0000	1.0000	0.0007	8.4000
CASE	63	2.0000	1.0000	1.0000	0.0006	7.1000
CASE	64	2.0000	1.0000	1.0000	0.0006	7.7000
CASE	65	2.0000	1.0000	1.0000	0.0007	7.5000
CASE	66	2.0000	1.0000	1.0000	0.0009	9.0000
CASE	67	2.0000	1.0000	1.0000	0.0007	7.6000
CASE	68	2.0000	1.0000	1.0000	0.0007	6.6000
CASE	69	2.0000	1.0000	1.0000	0.0006	7.4000
CASE	70	2.0000	1.0000	1.0000	0.0007	6.8000
CASE	71	2.0000	1.0000	1.0000	0.0008	8.4000
CASE	72	2.0000	1.0000	2.0000	0.0010	7.7000
CASE	73	2.0000	1.0000	2.0000	0.0008	7.5000
CASE	74	2.0000	1.0000	2.0000	0.0006	8.0000
CASE	75	2.0000	1.0000	2.0000	0.0008	7.5000
CASE	76	2.0000	1.0000	2.0000	0.0007	7.8000
CASE	77	2.0000	1.0000	2.0000	0.0008	7.5000
CASE	78	2.0000	1.0000	2.0000	0.0008	8.3000
CASE	79	2.0000	1.0000	2.0000	0.0010	7.5000
CASE	80	2.0000	1.0000	2.0000	0.0008	7.4000
CASE	81	2.0000	1.0000	2.0000	0.0009	7.5000
CASE	82	2.0000	1.0000	2.0000	0.0010	7.2000
CASE	83	2.0000	2.0000	1.0000	0.0009	7.9000
CASE	84	2.0000	2.0000	1.0000	0.0006	7.5000
CASE	85	2.0000	2.0000	1.0000	0.0007	7.0000
CASE	86	2.0000	2.0000	1.0000	0.0007	6.4000
CASE	87	2.0000	2.0000	1.0000	0.0007	7.1000
CASE	88	2.0000	2.0000	1.0000	0.0006	7.4000
CASE	89	2.0000	2.0000	1.0000	0.0008	6.6000
CASE	90	2.0000	2.0000	1.0000	0.0005	7.6000
CASE	91	2.0000	2.0000	1.0000	0.0005	7.7000
CASE	92	2.0000	2.0000	1.0000	0.0007	8.3000
CASE	93	2.0000	2.0000	1.0000	0.0007	8.0000
CASE	94	2.0000	2.0000	1.0000	0.0007	8.4000
CASE	95	2.0000	2.0000	1.0000	0.0006	7.5000
CASE	96	2.0000	2.0000	1.0000	0.0007	9.2000
CASE	97	2.0000	2.0000	2.0000	0.0009	7.3000
CASE	98	2.0000	2.0000	2.0000	0.0009	7.6000
CASE	99	2.0000	2.0000	2.0000	0.0008	6.8000
CASE	100	2.0000	2.0000	2.0000	0.0010	8.5000
CASE	101	2.0000	2.0000	2.0000	0.0009	7.5000
CASE	102	2.0000	2.0000	2.0000	0.0008	7.5000
CASE	103	2.0000	2.0000	2.0000	0.0009	7.4000
CASE	104	2.0000	2.0000	2.0000	0.0008	8.4000
CASE	105	2.0000	2.0000	2.0000	0.0008	8.0000
CASE	106	2.0000	2.0000	2.0000	0.0008	8.0000
CASE	107	2.0000	2.0000	2.0000	0.0006	7.8000
CASE	108	2.0000	2.0000	2.0000	0.0006	6.2000
CASE	109	2.0000	2.0000	2.0000	0.0007	7.8000
CASE	110	3.0000	1.0000	1.0000	0.0007	8.7000
CASE	111	3.0000	1.0000	1.0000	0.0008	8.3000
CASE	112	3.0000	1.0000	1.0000	0.0007	7.8000
CASE	113	3.0000	1.0000	1.0000	0.0009	8.5000
CASE	114	3.0000	1.0000	1.0000	0.0006	8.4000
CASE	115	3.0000	1.0000	1.0000	0.0009	7.3000
CASE	116	3.0000	1.0000	1.0000	0.0008	8.4000
CASE	117	3.0000	1.0000	1.0000	0.0007	7.4000
CASE	118	3.0000	1.0000	1.0000	0.0005	7.3000
CASE	119	3.0000	1.0000	1.0000	0.0006	7.4000
CASE	120	3.0000	1.0000	1.0000	0.0007	7.4000
CASE	121	3.0000	1.0000	1.0000	0.0007	8.4000
CASE	122	3.0000	1.0000	2.0000	0.0010	7.1000
CASE	123	3.0000	1.0000	2.0000	0.0010	7.7000
CASE	124	3.0000	1.0000	2.0000	0.0009	9.1000
CASE	125	3.0000	1.0000	2.0000	0.0010	8.5000
CASE	126	3.0000	1.0000	2.0000	0.0012	9.0000
CASE	127	3.0000	1.0000	2.0000	0.0009	9.4000
CASE	128	3.0000	1.0000	2.0000	0.0009	8.4000
CASE	129	3.0000	1.0000	2.0000	0.0011	8.4000

114

CASE	130	3.0000	1.0000	2.0000	0.0008	7.8000
CASE	131	3.0000	1.0000	2.0000	0.0009	8.4000
CASE	132	3.0000	1.0000	2.0000	0.0012	9.1000
CASE	133	3.0000	1.0000	2.0000	0.0013	9.4000
CASE	134	3.0000	1.0000	2.0000	0.0008	8.4000
CASE	135	3.0000	1.0000	2.0000	0.0008	7.9000
CASE	136	3.0000	2.0000	1.0000	0.0009	7.9000
CASE	137	3.0000	2.0000	1.0000	0.0009	7.0000
CASE	138	3.0000	2.0000	1.0000	0.0007	6.9000
CASE	139	3.0000	2.0000	1.0000	0.0006	7.5000
CASE	140	3.0000	2.0000	1.0000	0.0006	8.2000
CASE	141	3.0000	2.0000	1.0000	0.0008	7.8000
CASE	142	3.0000	2.0000	1.0000	0.0007	6.7000
CASE	143	3.0000	2.0000	1.0000	0.0006	8.4000
CASE	144	3.0000	2.0000	1.0000	0.0005	7.1000
CASE	145	3.0000	2.0000	1.0000	0.0009	7.6000
CASE	146	3.0000	2.0000	1.0000	0.0010	7.4000
CASE	147	3.0000	2.0000	2.0000	0.0009	7.8000
CASE	148	3.0000	2.0000	2.0000	0.0010	7.4000
CASE	149	3.0000	2.0000	2.0000	0.0005	8.3000
CASE	150	3.0000	2.0000	2.0000	0.0010	8.3000
CASE	151	3.0000	2.0000	2.0000	0.0007	8.2000
CASE	152	3.0000	2.0000	2.0000	0.0007	7.4000
CASE	153	3.0000	2.0000	2.0000	0.0012	7.8000
CASE	154	3.0000	2.0000	2.0000	0.0014	10.0000
CASE	155	3.0000	2.0000	2.0000	0.0007	10.0000
CASE	156	3.0000	2.0000	2.0000	0.0009	6.8000
CASE	157	3.0000	2.0000	2.0000	0.0008	7.9000
CASE	158	3.0000	2.0000	2.0000	0.0012	8.0000
CASE	159	3.0000	2.0000	2.0000	0.0009	8.5000
CASE	160	3.0000	2.0000	2.0000	0.0012	7.3000
CASE	161	3.0000	2.0000	2.0000	0.0012	7.8000
CASE	162	3.0000	2.0000	2.0000	0.0007	8.2000
CASE	163	4.0000	1.0000	1.0000	0.0006	7.4000
CASE	164	4.0000	1.0000	1.0000	0.0007	8.4000
CASE	165	4.0000	1.0000	1.0000	0.0005	7.6000
CASE	166	4.0000	1.0000	1.0000	0.0006	7.3000
CASE	167	4.0000	1.0000	1.0000	0.0007	6.6000
CASE	168	4.0000	1.0000	1.0000	0.0006	7.5000
CASE	169	4.0000	1.0000	1.0000	0.0005	7.4000
CASE	170	4.0000	1.0000	1.0000	0.0006	8.3000
CASE	171	4.0000	1.0000	1.0000	0.0007	7.5000
CASE	172	4.0000	1.0000	1.0000	0.0005	7.4000
CASE	173	4.0000	1.0000	1.0000	0.0008	7.0000
CASE	174	4.0000	1.0000	1.0000	0.0008	8.0000
CASE	175	4.0000	1.0000	1.0000	0.0006	7.5000
CASE	176	4.0000	1.0000	1.0000	0.0006	7.5000
CASE	177	4.0000	1.0000	1.0000	0.0007	8.1000
CASE	178	4.0000	1.0000	1.0000	0.0007	8.4000
CASE	179	4.0000	1.0000	1.0000	0.0007	7.2000
CASE	180	4.0000	1.0000	2.0000	0.0007	7.3000
CASE	181	4.0000	1.0000	2.0000	0.0008	7.5000
CASE	182	4.0000	1.0000	2.0000	0.0009	7.8000
CASE	183	4.0000	1.0000	2.0000	0.0011	7.7000
CASE	184	4.0000	1.0000	2.0000	0.0009	8.5000
CASE	185	4.0000	1.0000	2.0000	0.0011	8.5000
CASE	186	4.0000	1.0000	2.0000	0.0010	8.4000
CASE	187	4.0000	1.0000	2.0000	0.0011	8.2000
CASE	188	4.0000	1.0000	2.0000	0.0009	8.3000
CASE	189	4.0000	1.0000	2.0000	0.0008	7.5000
CASE	190	4.0000	1.0000	2.0000	0.0007	7.5000
CASE	191	4.0000	2.0000	1.0000	0.0010	6.7000
CASE	192	4.0000	2.0000	1.0000	0.0009	8.4000
CASE	193	4.0000	2.0000	1.0000	0.0009	8.5000
CASE	194	4.0000	2.0000	1.0000	0.0007	7.4000
CASE	195	4.0000	2.0000	1.0000	0.0007	7.4000
CASE	196	4.0000	2.0000	1.0000	0.0007	8.2000
CASE	197	4.0000	2.0000	1.0000	0.0008	7.8000
CASE	198	4.0000	2.0000	1.0000	0.0008	7.9000
CASE	199	4.0000	2.0000	1.0000	0.0009	8.3000
CASE	200	4.0000	2.0000	1.0000	0.0007	8.3000
CASE	201	4.0000	2.0000	1.0000	0.0006	8.3000

115
~~120~~

CASE	202	4.0000	2.0000	1.0000	0.0007	7.5000
CASE	203	4.0000	2.0000	1.0000	0.0008	7.9000
CASE	204	4.0000	2.0000	1.0000	0.0007	7.8000
CASE	205	4.0000	2.0000	2.0000	0.0012	8.0000
CASE	206	4.0000	2.0000	2.0000	0.0013	8.5000
CASE	207	4.0000	2.0000	2.0000	0.0009	8.5000
CASE	208	4.0000	2.0000	2.0000	0.0012	8.4000
CASE	209	4.0000	2.0000	2.0000	0.0012	8.4000
CASE	210	4.0000	2.0000	2.0000	0.0011	8.4000
CASE	211	4.0000	2.0000	2.0000	0.0009	8.3000
CASE	212	4.0000	2.0000	2.0000	0.0009	8.5000
CASE	213	4.0000	2.0000	2.0000	0.0012	8.7000
CASE	214	4.0000	2.0000	2.0000	0.0012	7.7000
CASE	215	4.0000	2.0000	2.0000	0.0014	8.7000
CASE	216	5.0000	1.0000	1.0000	0.0008	8.5000
CASE	217	5.0000	1.0000	1.0000	0.0006	7.3000
CASE	218	5.0000	1.0000	1.0000	0.0008	8.5000
CASE	219	5.0000	1.0000	1.0000	0.0006	7.5000
CASE	220	5.0000	1.0000	1.0000	0.0011	7.5000
CASE	221	5.0000	1.0000	1.0000	0.0006	7.8000
CASE	222	5.0000	1.0000	1.0000	0.0006	8.3000
CASE	223	5.0000	1.0000	1.0000	0.0005	7.6000
CASE	224	5.0000	1.0000	1.0000	0.0008	7.2000
CASE	225	5.0000	1.0000	1.0000	0.0009	8.4000
CASE	226	5.0000	1.0000	1.0000	0.0006	7.5000
CASE	227	5.0000	1.0000	1.0000	0.0007	7.5000
CASE	228	5.0000	1.0000	1.0000	0.0006	7.3000
CASE	229	5.0000	1.0000	1.0000	0.0008	7.8000
CASE	230	5.0000	1.0000	2.0000	0.0011	7.0000
CASE	231	5.0000	1.0000	2.0000	0.0009	7.3000
CASE	232	5.0000	1.0000	2.0000	0.0009	7.8000
CASE	233	5.0000	1.0000	2.0000	0.0008	7.7000
CASE	234	5.0000	1.0000	2.0000	0.0007	8.5000
CASE	235	5.0000	1.0000	2.0000	0.0010	8.4000
CASE	236	5.0000	1.0000	2.0000	0.0007	7.8000
CASE	237	5.0000	1.0000	2.0000	0.0008	7.8000
CASE	238	5.0000	1.0000	2.0000	0.0010	7.0000
CASE	239	5.0000	1.0000	2.0000	0.0009	8.6000
CASE	240	5.0000	1.0000	2.0000	0.0010	7.6000
CASE	241	5.0000	2.0000	1.0000	0.0009	7.7000
CASE	242	5.0000	2.0000	1.0000	0.0006	8.5000
CASE	243	5.0000	2.0000	1.0000	0.0005	6.4000
CASE	244	5.0000	2.0000	1.0000	0.0008	7.6000
CASE	245	5.0000	2.0000	1.0000	0.0005	7.5000
CASE	246	5.0000	2.0000	1.0000	0.0007	7.4000
CASE	247	5.0000	2.0000	1.0000	0.0006	8.4000
CASE	248	5.0000	2.0000	1.0000	0.0007	7.0000
CASE	249	5.0000	2.0000	1.0000	0.0007	8.3000
CASE	250	5.0000	2.0000	1.0000	0.0008	6.8000
CASE	251	5.0000	2.0000	1.0000	0.0008	7.7000
CASE	252	5.0000	2.0000	1.0000	0.0006	7.4000
CASE	253	5.0000	2.0000	1.0000	0.0008	7.3000
CASE	254	5.0000	2.0000	1.0000	0.0007	7.8000
CASE	255	5.0000	2.0000	2.0000	0.0013	8.4000
CASE	256	5.0000	2.0000	2.0000	0.0007	7.1000
CASE	257	5.0000	2.0000	2.0000	0.0010	8.0000
CASE	258	5.0000	2.0000	2.0000	0.0010	8.3000
CASE	259	5.0000	2.0000	2.0000	0.0008	7.6000
CASE	260	5.0000	2.0000	2.0000	0.0009	7.5000
CASE	261	5.0000	2.0000	2.0000	0.0007	8.4000
CASE	262	5.0000	2.0000	2.0000	0.0010	8.3000
CASE	263	5.0000	2.0000	2.0000	0.0009	7.6000
CASE	264	5.0000	2.0000	2.0000	0.0011	6.9000
CASE	265	5.0000	2.0000	2.0000	0.0007	8.4000
CASE	266	6.0000	1.0000	1.0000	0.0008	8.4000
CASE	267	6.0000	1.0000	1.0000	0.0007	7.6000
CASE	268	6.0000	1.0000	1.0000	0.0007	7.4000
CASE	269	6.0000	1.0000	1.0000	0.0007	8.0000
CASE	270	6.0000	1.0000	1.0000	0.0007	7.8000
CASE	271	6.0000	1.0000	1.0000	0.0007	8.2000
CASE	272	6.0000	1.0000	1.0000	0.0007	8.0000
CASE	273	6.0000	1.0000	1.0000	0.0006	7.4000

116

CASE	274	6.0000	1.0000	1.0000	0.0007	8.0000
CASE	275	6.0000	1.0000	1.0000	0.0007	8.4000
CASE	276	6.0000	1.0000	1.0000	0.0009	7.6000
CASE	277	6.0000	1.0000	1.0000	0.0008	7.6000
CASE	278	6.0000	1.0000	1.0000	0.0007	7.6000
CASE	279	6.0000	1.0000	2.0000	0.0007	6.8000
CASE	280	6.0000	1.0000	2.0000	0.0008	7.4000
CASE	281	6.0000	1.0000	2.0000	0.0008	7.5000
CASE	282	6.0000	1.0000	2.0000	0.0007	7.8000
CASE	283	6.0000	1.0000	2.0000	0.0008	6.8000
CASE	284	6.0000	1.0000	2.0000	0.0006	7.6000
CASE	285	6.0000	1.0000	2.0000	0.0009	8.3000
CASE	286	6.0000	1.0000	2.0000	0.0006	7.8000
CASE	287	6.0000	1.0000	2.0000	0.0008	7.5000
CASE	288	6.0000	2.0000	1.0000	0.0006	7.5000
CASE	289	6.0000	2.0000	1.0000	0.0008	7.3000
CASE	290	6.0000	2.0000	1.0000	0.0007	7.4000
CASE	291	6.0000	2.0000	1.0000	0.0005	8.0000
CASE	292	6.0000	2.0000	1.0000	0.0007	7.4000
CASE	293	6.0000	2.0000	1.0000	0.0006	7.5000
CASE	294	6.0000	2.0000	1.0000	0.0007	7.7000
CASE	295	6.0000	2.0000	1.0000	0.0006	7.4000
CASE	296	6.0000	2.0000	1.0000	0.0006	7.9000
CASE	297	6.0000	2.0000	1.0000	0.0007	7.0000
CASE	298	6.0000	2.0000	1.0000	0.0007	7.5000
CASE	299	6.0000	2.0000	1.0000	0.0007	8.6000
CASE	300	6.0000	2.0000	1.0000	0.0007	7.5000
CASE	301	6.0000	2.0000	2.0000	0.0010	7.7000
CASE	302	6.0000	2.0000	2.0000	0.0008	8.3000
CASE	303	6.0000	2.0000	2.0000	0.0007	7.8000
CASE	304	6.0000	2.0000	2.0000	0.0009	7.6000
CASE	305	6.0000	2.0000	2.0000	0.0008	7.2000
CASE	306	6.0000	2.0000	2.0000	0.0007	7.2000
CASE	307	6.0000	2.0000	2.0000	0.0006	8.3000
CASE	308	6.0000	2.0000	2.0000	0.0008	8.4000
CASE	309	6.0000	2.0000	2.0000	0.0010	8.0000
CASE	310	6.0000	2.0000	2.0000	0.0007	8.7000
CASE	311	6.0000	2.0000	2.0000	0.0010	7.5000
CASE	312	7.0000	1.0000	1.0000	0.0006	6.9000
CASE	313	7.0000	1.0000	1.0000	0.0006	6.5000
CASE	314	7.0000	1.0000	1.0000	0.0005	6.7000
CASE	315	7.0000	1.0000	1.0000	0.0003	6.5000
CASE	316	7.0000	1.0000	1.0000	0.0005	6.5000
CASE	317	7.0000	1.0000	1.0000	0.0004	7.0000
CASE	318	7.0000	1.0000	2.0000	0.0006	7.5000
CASE	319	7.0000	1.0000	2.0000	0.0009	7.7000
CASE	320	7.0000	1.0000	2.0000	0.0007	7.4000
CASE	321	7.0000	1.0000	2.0000	0.0008	7.4000
CASE	322	7.0000	1.0000	2.0000	0.0006	8.5000
CASE	323	7.0000	1.0000	2.0000	0.0006	8.4000
CASE	324	7.0000	1.0000	2.0000	0.0005	7.5000
CASE	325	7.0000	1.0000	2.0000	0.0008	7.2000
CASE	326	7.0000	1.0000	2.0000	0.0006	7.0000
CASE	327	7.0000	2.0000	1.0000	0.0005	6.9000
CASE	328	7.0000	2.0000	1.0000	0.0007	6.5000
CASE	329	7.0000	2.0000	1.0000	0.0006	7.0000
CASE	330	7.0000	2.0000	2.0000	0.0007	7.0000
CASE	331	7.0000	2.0000	2.0000	0.0006	6.4000
CASE	332	7.0000	2.0000	2.0000	0.0006	6.6000
CASE	333	7.0000	2.0000	2.0000	0.0008	7.4000

Carbendazim : Mysidopsis bahia
ANOVA on Male Weights
LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT
1.0000 2.0000 3.0000 4.0000 5.0000 6.0000
7.0000
REP
1.0000 2.0000

DEP VAR: WEIGHT N: 176 MULTIPLE R: 0.449 SQUARED MULTIPLE R: 0.202

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	0.0000	6	0.0000	3.1625	0.0058
REP	0.0000	1	0.0000	1.2269	0.2697
TRT*REP	0.0000	6	0.0000	2.7107	0.0155
ERROR	0.0000	162	0.0000		

Post-hoc pairwise comparison of weight/Bonferroni.
COL/
ROW TRT
1 1.0000
2 2.0000
3 3.0000
4 4.0000
5 5.0000
6 6.0000
7 7.0000

USING LEAST SQUARES MEANS.
POST HOC TEST OF WEIGHT

MATRIX OF PAIRWISE MEAN DIFFERENCES:

	1	2	3	4	5
1	0.0000				
2	0.0000	0.0000			
3	0.0001	0.0001	0.0000		
4	0.0000	0.0000	-0.0000	0.0000	
5	0.0000	0.0000	-0.0000	-0.0000	0.0000
6	0.0000	0.0000	-0.0000	-0.0000	-0.0000
7	-0.0001	-0.0001	-0.0002	-0.0002	-0.0002

	6	7
6	0.0000	
7	-0.0002	0.0000

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	1.0000	1.0000			
3	1.0000	1.0000	1.0000		
4	1.0000	1.0000	1.0000	1.0000	
5	1.0000	1.0000	1.0000	1.0000	1.0000
6	1.0000	1.0000	1.0000	1.0000	1.0000
7	0.1038	0.0804	0.0018	0.0061	0.0113

	6	7
6	1.0000	
7	0.0287	1.0000

Carbendazim : Mysidopsis bahia
ANOVA on Female Weights

LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT
1.0000 2.0000 3.0000 4.0000 5.0000 6.0000
7.0000
REP
1.0000 2.0000

DEP VAR: WEIGHT N: 157 MULTIPLE R: 0.561 SQUARED MULTIPLE R: 0.315

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	0.0000	6	0.0000	8.4840	0.0000
REP	0.0000	1	0.0000	1.1566	0.2840
TRT*REP	0.0000	6	0.0000	1.8708	0.0898
ERROR	0.0000	143	0.0000		

Post-hoc pairwise comparison of weight/Bonferroni.

COL/ ROW	TRT
1	1.0000
2	2.0000
3	3.0000
4	4.0000
5	5.0000
6	6.0000
7	7.0000

USING LEAST SQUARES MEANS.
POST HOC TEST OF WEIGHT

MATRIX OF PAIRWISE MEAN DIFFERENCES:

	1	2	3	4	5
1	0.0000				
2	-0.0001	0.0000			
3	0.0000	0.0001	0.0000		
4	0.0001	0.0002	0.0001	0.0000	
5	-0.0000	0.0001	-0.0001	-0.0001	0.0000
6	-0.0002	-0.0000	-0.0002	-0.0002	-0.0001
7	-0.0003	-0.0001	-0.0003	-0.0003	-0.0002

	6	7
6	0.0000	
7	-0.0001	0.0000

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	0.4068	1.0000			
3	1.0000	0.0640	1.0000		
4	1.0000	0.0019	1.0000	1.0000	
5	1.0000	1.0000	1.0000	0.4512	1.0000
6	0.0563	1.0000	0.0067	0.0002	0.4126
7	0.0007	0.3959	0.0001	0.0000	0.0071

	6	7
6	1.0000	
7	1.0000	1.0000

Carbendazim : Mysidopsis bahia

ANOVA on Male Lengths
LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT
1.0000 2.0000 3.0000 4.0000 5.0000 6.0000
7.0000
REP
1.0000 2.0000

DEP VAR: LENGTH N: 176 MULTIPLE R: 0.500 SQUARED MULTIPLE R: 0.250

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	12.0280	6	2.0047	7.0022	0.0000
REP	0.1534	1	0.1534	0.5360	0.4652
TRT*REP	2.3391	6	0.3899	1.3617	0.2331
ERROR	46.3792	162	0.2863		

Post-hoc pairwise comparison of length/Bonferroni.

COL/ ROW	TRT
1	1.0000
2	2.0000
3	3.0000
4	4.0000
5	5.0000
6	6.0000
7	7.0000

USING LEAST SQUARES MEANS.
POST HOC TEST OF LENGTH

MATRIX OF PAIRWISE MEAN DIFFERENCES:

	1	2	3	4	5
1	0.0000				
2	0.4252	0.0000			
3	0.5289	0.1037	0.0000		
4	0.5480	0.1228	0.0191	0.0000	
5	0.4687	0.0436	-0.0601	-0.0792	0.0000
6	0.5273	0.1021	-0.0016	-0.0207	0.0585
7	-0.4503	-0.8755	-0.9792	-0.9982	-0.9190

	6	7
6	0.0000	
7	-0.9776	0.0000

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	0.0567	1.0000			
3	0.0102	1.0000	1.0000		
4	0.0021	1.0000	1.0000	1.0000	
5	0.0226	1.0000	1.0000	1.0000	1.0000
6	0.0068	1.0000	1.0000	1.0000	1.0000
7	0.7570	0.0014	0.0003	0.0001	0.0007

	6	7
6	1.0000	
7	0.0003	1.0000

120
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Carbendazim : Mysidopsis bahia
ANOVA on Female Lengths
LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT

1.0000	2.0000	3.0000	4.0000	5.0000	6.0000
7.0000					

REP

1.0000	2.0000

DEP VAR: LENGTH N: 157 MULTIPLE R: 0.546 SQUARED MULTIPLE R: 0.298

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	13.9598	6	2.3266	7.3582	0.0000
REP	0.0832	1	0.0832	0.2631	0.6088
TRT*REP	6.3656	6	1.0609	3.3553	0.0040
ERROR	45.2157	143	0.3162		

Post-hoc pairwise comparison of length/Bonferroni.
COL/

ROW	TRT
1	1.0000
2	2.0000
3	3.0000
4	4.0000
5	5.0000
6	6.0000
7	7.0000

USING LEAST SQUARES MEANS.
POST HOC TEST OF LENGTH

MATRIX OF PAIRWISE MEAN DIFFERENCES:

	1	2	3	4	5
1	0.0000				
2	-0.1461	0.0000			
3	0.5291	0.6752	0.0000		
4	0.3903	0.5364	-0.1388	0.0000	
5	0.0585	0.2045	-0.4707	-0.3318	0.0000
6	-0.0688	0.0773	-0.5979	-0.4591	-0.1273
7	-0.5236	-0.3775	-1.0527	-0.9139	-0.5821

	6	7
6	0.0000	
7	-0.4548	0.0000

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	1.0000	1.0000			
3	0.0137	0.0005	1.0000		
4	0.3883	0.0327	1.0000	1.0000	
5	1.0000	1.0000	0.0713	1.0000	1.0000
6	1.0000	1.0000	0.0072	0.1958	1.0000
7	0.2250	1.0000	0.0000	0.0004	0.1187

	6	7
6	1.0000	
7	0.6886	1.0000

Carbendazim : Mysidopsis bahia

THE FOLLOWING RESULTS ARE FOR:
TRT = 1.0000

TOTAL OBSERVATIONS: 56

	WEIGHT	LENGTH
N OF CASES	56	56
MINIMUM	0.0004	6.2000
MAXIMUM	0.0013	9.2000
MEAN	0.0008	7.4354
STANDARD DEV	0.0002	0.6256

THE FOLLOWING RESULTS ARE FOR:
TRT = 2.0000

TOTAL OBSERVATIONS: 53

	WEIGHT	LENGTH
N OF CASES	53	53
MINIMUM	0.0005	6.2000
MAXIMUM	0.0010	9.2000
MEAN	0.0007	7.6151
STANDARD DEV	0.0001	0.5898

THE FOLLOWING RESULTS ARE FOR:
TRT = 3.0000

TOTAL OBSERVATIONS: 53

	WEIGHT	LENGTH
N OF CASES	53	53
MINIMUM	0.0005	6.7000
MAXIMUM	0.0014	10.0000
MEAN	0.0009	8.0396
STANDARD DEV	0.0002	0.7535

THE FOLLOWING RESULTS ARE FOR:
TRT = 4.0000

TOTAL OBSERVATIONS: 53

	WEIGHT	LENGTH
N OF CASES	53	53
MINIMUM	0.0005	6.6000
MAXIMUM	0.0014	8.7000
MEAN	0.0008	7.9019
STANDARD DEV	0.0002	0.5239

THE FOLLOWING RESULTS ARE FOR:
TRT = 5.0000

TOTAL OBSERVATIONS: 50

	WEIGHT	LENGTH
N OF CASES	50	50
MINIMUM	0.0005	6.4000
MAXIMUM	0.0013	8.6000
MEAN	0.0008	7.7300
STANDARD DEV	0.0002	0.5380

THE FOLLOWING RESULTS ARE FOR:
TRT = 6.0000

TOTAL OBSERVATIONS: 46

	WEIGHT	LENGTH
N OF CASES	46	46
MINIMUM	0.0005	6.8000
MAXIMUM	0.0010	8.7000
MEAN	0.0007	7.7152
STANDARD DEV	0.0001	0.4417

THE FOLLOWING RESULTS ARE FOR:
TRT = 7.0000

TOTAL OBSERVATIONS: 22

	WEIGHT	LENGTH
N OF CASES	22	22
MINIMUM	0.0003	6.4000
MAXIMUM	0.0009	8.5000
MEAN	0.0006	7.1136
STANDARD DEV	0.0001	0.5801

SUMMARY STATISTICS FOR WEIGHT

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 37.4442 DF= 6 PROBABILITY = 0.0000

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	0.0000	6	0.0000	6.6513	0.0000
WITHIN GROUPS	0.0000	326	0.0000		

SUMMARY STATISTICS FOR LENGTH

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 16.0578 DF= 6 PROBABILITY = 0.0134

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	20.1920	6	3.3653	9.6711	0.0000
WITHIN GROUPS	113.4412	326	0.3480		

SUMMARY STATISTICS FOR WEIGHT

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 56.5159 DF= 27 PROBABILITY = 0.0007

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	0.0000	27	0.0000	10.7443	0.0000
WITHIN GROUPS	0.0000	305	0.0000		

SUMMARY STATISTICS FOR LENGTH

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 39.3290 DF= 27 PROBABILITY = 0.0591

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	42.0382	27	1.5570	5.1845	0.0000
WITHIN GROUPS	91.5950	305	0.3003		

KOLMOGOROV-SMIRNOV ONE SAMPLE TEST USING STANDARD NORMAL DISTRIBUTION

VARIABLE	N-OF-CASES	MAXDIF	PROBABILITY (2-TAIL)
WEIGHT	333.0000	0.5001	0.0000
LENGTH	333.0000	1.0000	0.0000

DP Barcode : D204141
PC Code No : 099101
EEB Out :

To: Linda Propst/Susanne Cerrelli, PM 73
Special Review and Reregisration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 099101
Chemical Name : Benomyl
Type Product : Fungicide
Product Name :
Company Name : DuPont
Purpose : Registrant's request to reevaluate MATC for catfish early life-stage study (MRID #419457-01)
Action Code : 606 Date Due : 06/27/94
Reviewer : William A. Erickson

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.
71-1(a)			72-2(a)			72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)			122-1(a)		
71-2(b)			72-3(b)			122-1(b)		
71-3			72-3(c)			122-2		
71-4(a)			72-3(d)			123-1(a)		
71-4(b)			72-3(e)			123-1(b)		
71-5(a)			72-3(f)			123-2		
71-5(b)			72-4(a)			124-1		
72-1(a)			72-4(b)			124-2		
72-1(b)			72-5			141-1		
72-1(c)			72-6			141-2		
72-1(d)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

125

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

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Benomyl: DuPont's request to reevaluate MATC for catfish early life-stage study

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 6/23/94

TO: Linda Propst/Susanne Cerrelli, PM 73
Special Review and Reregistration Division (7508W)

In a letter dated May 26, 1994, R. A. Carver of DuPont requests that the Agency reevaluate the MATC determined for the catfish early life-stage study (MRID No. 419457-01). In the DER, EEB found growth effects at all dosages but accepted an MATC (geometric mean) of 0.38 ppb, which was the lowest dosage tested. Based on the reported detection limit of 0.23 ppb for benomyl, we felt that a lower MATC probably could not be reliably established.

DuPont argues that the MATC should be 3.6 ppb, based on analyzing the data using a nested ANOVA design, rather than the two-way ANOVA used by EEB. However, regardless of which statistical test is employed, the study itself is flawed because of the adverse effects of the solvent (DMF) on the catfish. As the testing laboratory reported and EEB confirmed from the data, the solvent had a significant negative impact on growth and survival when compared to the water dilution control. Therefore, comparing treatment effects to either the solvent control data or the pooled data from both control groups is not scientifically sound; deriving an MATC from such data is not acceptable.

EEB derived an MATC by comparing growth and survival of the treated groups to that reported for the water dilution control group. This is a conservative approach, because it attributes all effects to the benomyl and none to the solvent. However, because the study is flawed, there is no way to separate effects of the solvent from those of the benomyl. DuPont has the option of accepting the lowest dosage tested (0.38 ppb) as the MATC or

126

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repeating the study. If the study is repeated, caution should be taken to ensure that the solvent has no signicant impact on hatching, survival, or growth.

DuPont also argues that means for fish length are only significant to the nearest millimeter, because the catfish were only measured to that level. However, the testing laboratory deviated from acceptable protocols by measuring only to the nearest millimeter; length should be measured to the nearest 0.5 mm. This correction, and other deviations noted in the DER, should be made if the study is repeated.

If you have any questions, please contact Bill Erickson at 305-6212 or Henry Craven at 305-5320.

127

T32

DP Barcode : D166853
PC Code No : 099101
EEB Out :

To: Linda Propst, PM 73
Special Review and Reregisration Division (7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 099101
Chemical Name : Benomyl
Type Product : Fungicide
Product Name :
Company Name : DuPont
Purpose : Catfish early life-stage toxicity test

Action Code : 627 Date Due : 11/21/91
Reviewer : William A. Erickson

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.
71-1(a)			72-2(a)			72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)			122-1(a)		
71-2(b)			72-3(b)			122-1(b)		
71-3			72-3(c)			122-2		
71-4(a)			72-3(d)			123-1(a)		
71-4(b)			72-3(e)			123-1(b)		
71-5(a)			72-3(f)			123-2		
71-5(b)			72-4(a)	419457-01	Y	124-1		
72-1(a)			72-4(b)			124-2		
72-1(b)			72-5			141-1		
72-1(c)			72-6			141-2		
72-1(d)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

128
133

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl: catfish early life-stage study

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Henry T. Craven 2/18/94

TO: Linda Propst, PM 73
Special Review and Reregistration Division (7508W)

Based on the information provided by Ronald A. Hamlen of du Pont in his letter dated July 15, 1991, EEB is upgrading to Core the study entitled "Early Life Stage Toxicity of ^{14}C-Benomyl to Channel Catfish (*Ictalurus punctatus*) in a Flow-through System." (MRID No. 419457-01). The study was submitted to fulfill the guideline requirement 72-4a for technical benomyl. The results and classification of this study are found in the attached DER.

If you have any questions, please contact Bill Erickson at 305-6212 or Henry Craven at 305-5320.

129

134

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DATA EVALUATION RECORD

1. **CHEMICAL:** Benomyl.
Shaughnessey No. 099101.

2. **TEST MATERIAL:** (1) ^{14}C-Benomyl, Lot No. T-1991, 96.7% purity, specific activity of 4.08 x 10^4 dpm/µg, a white powder; (2) Non-radiolabelled Benomyl, Lot No. T1991-479, 99.3% purity, a white powder.

3. **STUDY TYPE:** Fish Early Life-Stage Toxicity Test.
Species Tested: Channel Catfish (*Ictalurus punctatus*).

4. **CITATION:** McAllister, W.A. 1991. Early Life Stage Toxicity of ^{14}C-Benomyl to Channel Catfish (*Ictalurus punctatus*) in a Flow-Through System. Conducted by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. ABC Amended Final Report No. 36926. Submitted by E.I. du Pont de Nemours and Company, Inc., Newark, Delaware. Du Pont HLO 766-88. EPA MRID No. 419457-01.

5. **REVIEWED BY:**

William A. Erickson
Biologist
EEB/EFED

Signature: W. Erickson
Date: 2/18/94

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
EEB/EFED

Signature: Henry T. Craven
Date: 2/18/94

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement (72-4a) for a fish early life-stage toxicity test using the channel catfish. Fish length at all test levels was significantly reduced when compared to the dilution water control. The LOEC was determined to be 0.38 µg/l mean measured concentration (excluding day-21 measurement), the lowest level tested. The MATC of ^{14}C-benomyl for length, the most sensitive parameter, could not be determined from this study but was <0.38 µg/l. It is not likely that a lower MATC could be established as it may be below the limits of detection of benomyl. The MATC (geometric mean) for weight was 1.02 µg/l (>0.67 µg/l, <1.38 µg/l) and for survival was 3.85 µg/l (>2.50 µg/l, <5.20 µg/l).

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Three egg masses (<24 hours old) from 3 female channel catfish (*Ictalurus punctatus*) were obtained from Osage Catfisheries, Osage Beach, Missouri. The egg masses were gently pulled apart into small clumps (3-6 eggs each) and the clumps were composited into a glass dish for distribution into various test chambers.

B. **Test System:** A two-liter proportional diluter, with a 50% dilution factor, was used to intermittently deliver test solutions to test chambers (four chambers per concentration). Flow-splitting cells divided each of the five toxicant concentrations, the solvent control, and the control solutions into the respective test chambers.

Each duplicate glass aquarium was divided into two test chambers. The chamber measured approximately 15.7 X 30.5 cm with a water depth of 24 cm, yielding an approximate 11.5-liter volume. Each test chamber drain was covered with a 40-mesh stainless steel screen to prevent escape of the fish fry.

Egg incubation cups (20 cm tall, 9 cm in diameter) were made from 1-liter narrow mouth polyethylene bottles in which the bottoms had been removed. Stainless steel screen (16-mesh) was attached by melting the mesh into the plastic near the neck of the bottle. A rectangular hole (4 x 9 cm) was cut into the side of the bottle for water circulation purposes and was also covered with 16-mesh stainless steel screen. The cups were inverted and suspended in their respective test chambers. Air from an airstone inside the cup vigorously rolled the egg masses. The minimum water depth in the incubation cup was at least 14 cm.

Aerated test dilution water was delivered to the test chambers at an average rate of approximately 64 ml/minute/replicate, providing an average of 8 volume replacements in a 24-hour period. The test chambers were placed in a temperature controlled water bath maintained at 25 ±1°C. During the test, the eggs and

131

136

juveniles were on a 16-hour light photoperiod with the light intensity of 90 ±8 footcandles. All lights above the test system were off during the hatching period, except during daily observations. Eggs were shielded from any excess U.V. light by the use of both incandescent and fluorescent lights which emitted minimal wavelengths of the U.V. light spectrum.

The dilution water was a mixture of well water and reverse osmosis well water with the following characteristics: a Ph of 7-8, a specific conductivity of 123-162 μmhos/cm, and a total hardness and alkalinity of 42-46 and 40-52 mg/l as $CaCO_3$, respectively.

A total of 127.9 mg ^{14}C-Benomyl was mixed with 397.4 mg non-radiolabeled Benomyl, resulting in a specific activity of 9930 dpm/μg. The stock solution was prepared by dissolving 74-75 mg of the ^{14}C-Benomyl mixture in 20 ml of DMF.

C. **Dosage:** Forty-day, flow-through, early life-stage test. Based on a 10-day preliminary test, five nominal concentrations (0.30, 0.60, 1.2, 2.4, and 4.8 μg/l) were selected for the definitive test. A solvent control and a dilution water control were also tested concurrently.

D. **Design:** Each concentration, solvent control, and dilution water control were replicated four times. During the first 22 days of the test, the solvent control concentration was 12 μl/l DMF which was equivalent to the highest concentration of solvent in any of the test solutions. On day 23 of the test, the solvent concentration was reduced to 6.2 μl/l DMF.

The test was initiated when twenty eggs were impartially distributed (5 at a time) into each incubation cup (i.e., 80 eggs/concentration). Eggs and embryos were observed daily and dead eggs were removed to prevent fungal growth. When hatching commenced, the number of eggs hatched in each incubation cup was recorded daily until the hatching was complete (>95% hatched) on day 8 of the study. On day 8, the fry in each replicate were impartially thinned to 15 fry (i.e., 60 fry/concentration) and were released into the test chambers. The fry were observed daily.

From the beginning of the hatching phase until the termination of the study, all fish were fed a combination of live brine shrimp nauplii and a standard commercial dry fish food *ad libitum* two to three times a day. All test chambers were siphoned at least 4 times a week to remove fecal material and excess food.

Abnormal (sub-lethal) behavioral and physical changes as well as mortality were monitored by visually inspecting each growth chamber daily. Fish were not fed 24 hours prior to study termination. At test termination on day 40, all surviving fish were sacrificed and measured for standard length (to the nearest millimeter) and weight (to the nearest milligram).

Temperature, dissolved oxygen concentration (DO), conductivity, and pH were measured on day 0, 1, 7, 14, 21, 28, 35, and 40. DO and temperature were measured in each replicate chamber having surviving eggs or fry on the designated sampling days. In addition, the temperature was measured twice daily and continuously recorded with a data logger in a centrally located test chamber. Conductivity, pH, hardness, and alkalinity were measured in the control, lowest and highest test concentrations having surviving embryos or fry.

The concentrations of ^{14}C-Benomyl in test solutions were measured using liquid scintillation counting (LSC) on days 0, 1, 7, and every 7 days thereafter until test termination on day 40.

E. **Statistics:** The experimental units were individual fish for continuous data (e.g., growth measurements) or the replicated chamber for dichotomous data (e.g., number hatching or surviving).

Solvent and dilution water controls were compared using a t-test for continuous data and a 2 x 2 contingency table for dichotomous data. If the result was a rejection of equality or independence, then only the solvent control was used for comparison to the treatments. When the hypothesis of equality was not rejected, data from only the control was used for comparison to the treatments.

Statistical analyses, appropriate for a nested experimental design, were performed on pooled replicate data using 2 x 2 contingency tables for dichotomous

data and analysis of variance techniques (ANOVA) coupled with Tukey's HSD test for continuous data. Data analyses also included the Bartlett's test for homogeneity of group variances by replicate for both weight and length measurements.

All statistical decisions were based upon a 0.05 level of significance.

12. **REPORTED RESULTS:** The mean measured concentrations of ^{14}C-Benomyl were 0.422, 0.712, 1.44, 2.67, and 5.57 µg/l, representing 141, 119, 120, 111, and 116% of the nominal values, respectively (Table 4, attached). Average values of water quality parameters measured during the test are shown in Tables 6 and 7 (attached). The DO levels ranged from 3.8 to 8.8 mg/l, which represented 48 and 111% saturation at 25°C. Oxygen saturation less than 75% occurred during week 5 and did not appear to cause any problems. The temperature remained at an average of 25°C and did not fluctuate by more than ±1°C in any 24-hour period.

A statistically significant DMF effect, at 12 µl/l, was detected as determined by percent hatch, weight, and length. Therefore, subsequent statistical analysis for all study parameters were made using the solvent control fish, although no change in concentrations that were significantly different occurred when combining the control and solvent control data. "Due to the design of the diluter used for this study only the solvent control and the highest test concentration had an equal solvent level. All lower treatment levels have proportionally 50% lower solvent concentrations. Therefore, a statistical difference between the control and solvent control is only relevant to the high test concentration."

Daily biological observations on the channel catfish eggs and fry appeared to show no morphological or behavioral abnormalities related to the ^{14}C-Benomyl in any of the test levels. Hatchability of eggs was significantly lower in only the highest test concentration (i.e., 5.57 µg/l) when compared with the hatching of either the control or solvent control eggs (Table 9, attached). Hatching occurred between days 5 and 8, with no apparent difference in time to hatching between test levels. Also, there was no apparent difference between the appearance of the control eggs and those continuously exposed to the four lowest ^{14}C-Benomyl test concentrations. Time to swim-up is not appropriate for a channel catfish study, therefore, no results for this parameter were obtained.

1134

134

By day 19 post-hatch, all fish in the highest test concentration (5.57 µg/l) had died. Survival was not significantly affected in the four lowest test concentrations when compared to the control or solvent control (Tables 9 and 10, attached).

Length and wet weight at test termination were not significantly reduced at any of the four lowest test concentrations (Tables 9 and 10, attached). Although the middle test level of 1.44 µg/l had a statistically significant reduced length, it was not considered biologically significant since the data did not follow a typical dose-response relationship. Lengths and weights of channel catfish fry exposed to the highest test concentration of 5.57 µg/l were unavailable due to complete mortality and thus not used in the statistical analysis.

Based on the survival, length, and weight data, the maximum acceptable toxicant concentration (MATC) was between the mean measured ^{14}C-Benomyl concentrations of 2.67 µg/l and 5.57 µg/l (geometric mean MATC = 3.86 µg/l as ^{14}C-Benomyl). The no-observed-effect concentration (NOEC) was 2.67 µg/l.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

The study was audited by the laboratory's Quality Assurance Unit on several occasions to assure compliance with the EPA Good Laboratory Practice Standards; Pesticide Programs (40 CFR 160). A GLP compliance statement was included and signed by the Study Director.

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

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

The DO was as low as 3.8 mg/l or 48% of saturation during week 5 (day-35 measurements).

Each egg-incubation cup within test chambers was aerated with an air stone. The SEP states that test tanks and embryo cups should not be aerated. However, for channel catfish, ASTM (1988) recommends the cups be aerated at a rate sufficient to provide adequate water exchange and to vigorously roll the egg masses and keep them suspended in the water column. Therefore, the aeration in this study is acceptable.

ASTM states that no attempt should be made to remove dead embryos since the embryos of channel catfish are held together by an adhesive gelatinous matrix. It was reported in this study that dead eggs were removed at each daily observation.

ASTM recommends that each test chamber receive approximately 100 ml/minute of test solution. In this study, an average rate of 64 ml/minute/chamber was used.

The measured concentration of test material in four out of five treatment levels was more than 30% higher than the time-weighted average concentration for more than 5% of the duration of the test (see Table 4, attached).

The length of the fry was measured to the nearest millimeter. ASTM recommends the measurement to the nearest 0.5 mm.

B. **Statistical Analysis:** KBN's reviewer analyzed hatching and survival data using ANOVA after arcsine square-root transformation of the data. The data transformation was based on the method recommended by EPA (1988). Comparisons between the controls and each treatment level were performed using multiple comparison tests. All results confirm the statistical analyses performed by the author. Only hatching and survival at Level 5 were significantly reduced when compared to the dilution water control and solvent control groups.

In order to separate the replicate effects, if any, from the benomyl effects, length and weight data were analyzed using a two-way ANOVA, followed by the Bonferroni's test. Both weight and length of the solvent control fish were significantly lower than those of the control fish. In this study, the solvent concentration was not the same in all treatments (the four lowest treatment levels had less solvent than the solvent control) and the range of solvent concentration used affected the fish growth. Therefore, growth of each treatment group should be compared to that of the dilution water control group. The fish exposed to $\geq$1.44 μg/l benomyl concentrations had significantly lower weight than the control fish. Fish length in the solvent control and all treatment groups was significantly reduced when compared to that of the control group. All printouts are attached.

136

T-11

C. **Discussion/Results:** According to ASTM, the test is not acceptable if the measured concentration of test material in any treatment is more than 30% higher than the time-weighted average concentration for more than 5% of the duration of the test. In this test, four out of five treatment levels (Levels 1, 2, 4, and 5) exceeded the 5% limit. However, as Dupont pointed out in a letter to EPA (dated July 15, 1991), the concentrations tested were extremely low (range 0.3-4.8 ppb nominal) and excluding the concentrations measured on day 21 would result in a more conservative MATC. The measured concentrations in this test are therefore accepted. Consequently, the exposure concentrations (i.e., mean measured concentrations excluding day-21 values) are defined as 0.383, 0.674, 1.38, 2.50, and 5.20 µg/l.

This study is scientifically sound and fulfills the guideline requirements for an early life-stage toxicity test using channel catfish (*Ictalurus punctatus*). The MATC of ^{14}C-Benomyl for length, the most sensitive parameter, could not be determined from this study due to the significant decrease in length at all treatment levels. The lowest-observed-effect concentration (LOEC) was 0.383 µg/l mean measured concentration (excluding day-21 measurement); Therefore, the MATC for length is <0.383 µg/l. It is not likely that a lower MATC could be established as it may be below the limits of detection of benomyl. The MATC (geometric mean) for weight was 1.02 µg/l (>0.674 µg/l, <1.38 µg/l) and for survival was 3.85 µg/l (>2.50 µg/l, <5.20 µg/l).

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, June 15, 1992.

REFERENCE:

U.S. Environmental Protection Agency. 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. EPA/600/4-87/028. Pages 332-334.

137

~~42~~

EEB Review dated 5/4/1994 Benomyl

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Analysis of Variance File: hatch Date: 06-08-1992

N's, means and standard deviations based on dependent variable: HATCH

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean	S.D.
*		28	1.2110	0.2813
1	Control	4	1.4588	0.0000
2	Solvent control	4	1.3212	0.0481
3	0.422 µg/l	4	1.2186	0.1024
4	0.712 µg/l	4	1.3212	0.0481
5	1.44 µg/l	4	1.2971	0.0556
6	2.67 µg/l	4	1.2546	0.1765
7	5.57 µg/l	4	0.6054	0.2163

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance Dependent variable: HATCH

Source	df	SS (H)	MSS	F	P
Between Subjects	27	2.1358			
C (CONC)	6	1.8473	0.3079	22.417	0.0000
Subj w Groups	21	0.2884	0.0137		

Analysis of Variance File: hatch Date: 06-08-1992

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.459	6	1.255
2	1.321	7	0.605
3	1.219		
4	1.321		
5	1.297		

Comparison	Tukey-A*	Newman -Keuls*	Dunnett
1 > 2			
1 > 3			0.0500
1 > 4			
1 > 5			
1 > 6			
1 > 7	0.0100	0.0100	0.0100
2 > 3			N.A.
2 = 4			N.A.
2 > 5			N.A.
2 > 6			N.A.
2 > 7	0.0100	0.0100	N.A.
3 < 4			N.A.
3 < 5			N.A.
3 < 6			N.A.
3 > 7	0.0100	0.0100	N.A.
4 > 5			N.A.
4 > 6			N.A.
4 > 7	0.0100	0.0100	N.A.
5 > 6			N.A.
5 > 7	0.0100	0.0100	N.A.
6 > 7	0.0100	0.0100	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500). A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Variance File: survival Date: 06-08-1992

N's, means and standard deviations based on dependent variable: SURVIVAL

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean	S.D.
*		28	1.2111	0.4598
1	Control	4	1.4084	0.0659
2	Solvent control	4	1.3802	0.1222
3	0.422 µg/l	4	1.3802	0.1222
4	0.712 µg/l	4	1.3802	0.1222
5	1.44 µg/l	4	1.4413	0.0000
6	2.67 µg/l	4	1.3577	0.1671
7	5.57 µg/l	4	0.1295	0.0000

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance Dependent variable: SURVIVAL

Source	df	SS (H)	MSS	F	P
Between Subjects	27	5.7074			
C (CONC)	6	5.4763	0.9127	82.953	0.0000
Subj w Groups	21	0.2311	0.0110		

145

150

Analysis of Variance File: survival Date: 06-08-1992

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.408	6	1.358
2	1.380	7	0.130
3	1.380		
4	1.380		
5	1.441		

Comparison	Tukey-A*	Newman -Keuls*	Dunnett
1 > 2			
1 > 3			
1 > 4			
1 < 5			
1 > 6			
1 > 7	0.0100	0.0100	0.0100
2 = 3			N.A.
2 < 4			N.A.
2 < 5			N.A.
2 > 6			N.A.
2 > 7	0.0100	0.0100	N.A.
3 < 4			N.A.
3 < 5			N.A.
3 > 6			N.A.
3 > 7	0.0100	0.0100	N.A.
4 < 5			N.A.
4 > 6			N.A.
4 > 7	0.0100	0.0100	N.A.
5 > 6			N.A.
5 > 7	0.0100	0.0100	N.A.
6 > 7	0.0100	0.0100	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500). A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Variance File: benomyl Date: 06-08-1992

N's, means and standard deviations based on dependent variable: WEIGHT

* Indicates statistics are collapsed over this factor

Factors: C	R		N	Mean	S.D.
*	*		350	733.8514	130.2899
1	*	Control	59	776.3220	122.8890
2	*	Solvent control	58	706.6724	120.5048
3	*	0.422 µg/l	58	762.1724	126.6207
4	*	0.712 µg/l	58	769.3621	148.6991
5	*	1.44 µg/l	60	698.2167	120.3752
6	*	2.67 µg/l	57	690.1053	114.7566
*	1		85	735.6588	147.1686
*	2		88	722.1477	144.9114
*	3		88	737.0795	116.8453
*	4		89	740.5056	110.4317
1	1		15	773.0667	168.9393
1	2		15	774.1334	122.1339
1	3		15	786.0000	91.5158
1	4		14	771.7857	106.8149
2	1		15	625.0000	101.4439
2	2		13	716.0769	94.4594
2	3		15	759.8000	147.2710
2	4		15	727.0667	93.7904
3	1		13	822.6154	135.7065
3	2		15	757.0667	131.2067
3	3		15	781.4667	72.2623
3	4		15	695.6000	135.9652
4	1		15	840.8000	119.8417
4	2		15	810.4667	168.2502
4	3		13	656.0769	113.1470
4	4		15	755.0000	130.2520
5	1		15	685.0667	88.0742
5	2		15	599.1334	129.8465
5	3		15	742.8000	95.5788
5	4		15	765.8666	97.8584
6	1		12	664.8333	127.0825
6	2		15	675.2000	119.9138
6	3		15	685.5333	123.4717
6	4		15	729.8000	88.6930

Fmax for testing homogeneity of between subjects variances: 5.47
Number of variances= 24 df per variance= 14.

Analysis of Variance Dependent variable: WEIGHT

Source	df	SS (H)	MSS	F	P
Between Subjects	349	5924431.5000			
C (CONC)	5	454197.4400	90839.4840	6.377	0.0000
R (REP)	3	19404.0352	6468.0117	0.454	0.7171
CR	15	807338.1200	53822.5430	3.779	0.0000
Subj w Groups	326	4643492.0000	14243.8408		

Analysis of Variance File: benomyl Date: 06-08-1992

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	776.322	6	690.105
2	706.672		
3	762.172		
4	769.362		
5	698.217		

1 = control
2 = solvent control
3 = 0.422 µg/l
4 = 0.712 "
5 = 1.44 "
6 = 2.67 "

Comparison	Bon-ferroni	Dunnett
1 > 2	0.0264	0.0100
1 > 3		
1 > 4		
1 > 5	0.0064	0.0100
1 > 6	0.0020	0.0100
2 < 3		N.A.
2 < 4		N.A.
2 > 5		N.A.
2 > 6		N.A.
3 < 4		N.A.
3 > 5		N.A.
3 > 6	0.0202	N.A.
4 > 5	0.0202	N.A.
4 > 6	0.0066	N.A.
5 > 6		N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Post-hoc tests for factor R (REP)

Level	Mean
1	735.659
2	722.148
3	737.080
4	740.506

Comparison	Bon-ferroni	Dunnett
1 > 2		
1 < 3		
1 < 4		
2 < 3		N.A.
2 < 4		N.A.
3 < 4		N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Variance File: benomyl Date: 06-08-1992

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors: C	R		N	Mean	S.D.
*	*		350	35.8229	2.3885
1	*	Control	59	37.2712	1.9194
2	*	Solvent control	58	35.8103	2.0040
3	*	0.422 µg/l	58	35.9138	2.3567
4	*	0.712 µg/l	58	36.0690	2.6282
5	*	1.44 µg/l	60	34.4833	2.4040
6	*	2.67 µg/l	57	35.4035	2.1201
*	1		85	36.1529	2.3730
*	2		88	35.5795	2.6683
*	3		88	35.7045	2.3152
*	4		89	35.8652	2.1752
1	1		15	37.4000	2.5298
1	2		15	36.9333	1.9074
1	3		15	37.2000	1.5213
1	4		14	37.5714	1.6968
2	1		15	34.6000	2.2615
2	2		13	36.1538	1.6756
2	3		15	36.5333	2.0656
2	4		15	36.0000	1.5119
3	1		13	37.3846	2.0631
3	2		15	36.0667	2.0517
3	3		15	36.8667	1.1872
3	4		15	33.5333	2.0656
4	1		15	37.3333	1.7182
4	2		15	36.7333	2.5765
4	3		13	33.3077	2.1750
4	4		15	36.5333	2.2318
5	1		15	35.3333	1.7182
5	2		15	32.2667	2.7377
5	3		15	34.8667	1.9591
5	4		15	35.4667	1.6847
6	1		12	34.7500	2.0057
6	2		15	35.4000	2.0284
6	3		15	35.1333	2.5317
6	4		15	36.2000	1.7809

Fmax for testing homogeneity of between subjects variances: 5.32
Number of variances= 24 df per variance= 14.

Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	349	1991.0184			
C (CONC)	5	245.4466	49.0893	12.002	0.0000
R (REP)	3	15.2287	5.0762	1.241	0.2922
CR	15	396.9915	26.4661	6.471	0.0000
Subj w Groups	326	1333.3517	4.0900		

Analysis of Variance File: benomyl Date: 06-08-1992

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	37.271	6	35.404
2	35.810		
3	35.914		
4	36.069		
5	34.483		

1 = control
2 = Solvent control
3 = 0.422 µg/l
4 = 0.712 "
5 = 1.44 "
6 = 2.67 "

Comparison	Bon-ferroni	Dunnett
1 > 2	0.0019	0.0100
1 > 3	0.0052	0.0100
1 > 4	0.0218	0.0100
1 > 5	0.0000	0.0100
1 > 6	0.0000	0.0100
2 < 3		N.A.
2 < 4		N.A.
2 > 5	0.0065	N.A.
2 > 6		N.A.
3 < 4		N.A.
3 > 5	0.0024	N.A.
3 > 6		N.A.
4 > 5	0.0005	N.A.
4 > 6		N.A.
5 < 6		N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Post-hoc tests for factor R (REP)

Level	Mean
1	36.153
2	35.580
3	35.705
4	35.865

Comparison	Bon-ferroni	Dunnett
1 > 2		
1 > 3		
1 > 4		
2 < 3		N.A.
2 < 4		N.A.
3 < 4		N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).