DATA EVALUATION SHEET

1. CHEMICAL: Bravo 500

2. FORMULATION: Chlorothalonil

3. CITATION


4. REVIEWED BY: Daniel Rieder
Wildlife Biologist
EEB/HED

5. DATE REVIEWED: March 20, 1980

6. TEST TYPE: Chronic Toxicity to Fish

   A. Test Species: Fathead Minnow (Pimephales)

   B. Test Material: Chlorothalonil (Technical, 96%)

7. REPORTED RESULTS

The maximum acceptable toxicant concentration (MATC), based on the effects of chlorothalonil on one generation (egg to egg) of fathead minnows, was estimated to be $3.0 < 6.5$ ppb. A flow through acute toxicity test was conducted with an estimated 96-hour $LC_{50}$ of 23 ppb (measured concentration).

8. REVIEWERS CONCLUSION

   A. Validation Category: Core

   B. Discussion

This study was scientifically conducted and fulfills the requirements for a chronic toxicity test for fish. Based on the estimated MATC and the acute toxicity test results, chlorothalonil is very highly toxic to fathead minnows.
METHODS/RESULTS

A. Test Procedures:

This test followed the "Recommended Bioassay Procedure for Fathead Minnow (Pimphales promelas Rafinesque) Chronic Tests". The following are exceptions to the referenced guidelines:

1. The test concentrations were measured weekly rather than daily.

2. The fry were photographed to determine total lengths and percentage survival on days 35 and 64 rather than days 30 and 60.

3. Temperature was measured weekly rather than daily.

4. The fry used in the acute toxicity test were 4 - 8 days old rather than the recommended age of 2 to 3 months.

Acetone was used as solvent, a solvent control test was conducted. Two test containers (replicates) were used at each test concentration level.

B. Statistical Analysis

1. Data for survival, growth and reproduction were subjected to a one-way analysis of variance (P=0.05). Percentage survival and percentage hatching success were transformed to the arcsin of the square root of the percentage prior to analysis of variance. If treatment effects were indicated, the treatment means for that parameter were compared to the control and the solvent control means by Dunnett's procedure using one-sided comparison.

2. The 96-hour IC\textsubscript{50} value and 95% confidence limits were calculated using the moving average method.

C. Results

1. The acute toxicity test resulted in a 96-hour IC\textsubscript{50} of 23 ppb with 20 to 26 ppb 95% confidence limits. The 35-day IC\textsubscript{50} and 95% confidence limits were 23 (19-26) ppb, virtually the same as the 96-hour value.

2. All F\textsubscript{0} fry in the A replicate of the solvent control died between days 16 and 33. This isolated incidence of mortality among fish exposed to acetone was attributed to a filamentous growth that occurred in spite of frequent cleanings. On day 64,
15 fish were transferred from B replicate of the solvent control to the A replicate aquarium and the test was continued with data from the duplicate solvent controls based on the transferred fish.

3. On day 168, 5 male fish in 4 different containers contracted gas bubble disease. This was attributed to the lower temperature of the inflowing water. The water was, from then on, heated to the level of the test containers before introduction into the aquariums and no more incidences of that disease were observed.

4. Hatching success of F₀ generation eggs was significantly reduced by exposure to a mean measured concentration of 16 ppb of chlorothalonil when compared to the control and solvent control. Thirty days after hatching, survival of F₀ fry exposed to 16 ppb was significantly reduced as compared to the controls. Growth was similar in all test containers throughout the test.

5. Most of the deaths in the F₀ generation occurred before day 35.

6. The number of eggs per spawn of F₀ minnows in the 16 and 6.5 ppb concentration was significantly less than in the controls.

7. Second generation (F₁ minnows were apparently more sensitive to chlorothalonil than were first generation (F₀) eggs. The percentage hatching success of F₁ eggs was significantly reduced, as compared to the controls, by exposure to a mean measured concentration of 6.5 ppb. This concentration did not significantly reduce the hatching of F₀ eggs. There was a statistically significant difference between the hatching success of F₀ eggs and F₁ eggs in the 6.5 ppb test concentration.

8. Summary

Chronic exposure of fathead minnows to concentrations of 16 and 6.5 ppb chlorothalonil produced adverse effects on egg production and on the survival of fish during early life stages, as well as indicating a possible cumulative affect from one generation of exposed fish to the next.

REVIEWERS EVALUATION

A. Test Procedure

The test procedures followed closely the EPA guidelines. The differences mentioned above were analyzed, and deemed acceptable.
B. Statistical Analysis

The following responses were compared, treatment to control, using a one-sided ANOVA and the Duncan’s Multiple Range Test:

1. Hatching success of $F_0$ generation eggs.
2. Survival of $F_0$ generation fry at 35 days.
3. Survival of $F_0$ generation fry at 64 days.
4. Number of spawns of $F_0$ generation fish.
5. Number of $F_1$ eggs per spawn of $F_0$ generation fish.
6. Hatching success of $F_1$ generation fry at 34 days.

The results of these analysis are provided in the attachment with the original report.

C. Results

The statistical analysis results indicate that chlorothalonil significantly reduces hatching success and survivability of fathead minnows at concentrations as low as 6.5 ppb. The maximum acceptable toxicant concentration (MATC) of technical chlorothalonil in water for fathead minnows is estimated to be in the range of 3.0 to 6.5 ppb. Other noteworthy results of the test are:

1. Most deaths occurred before the day 35 in all concentrations. This could mean that either the young fish are less tolerant, or the range of tolerance is great between fish and those fish not affected initially have some innate resistance to the toxicant and are unaffected.

2. The hatching success of $F_1$ generation eggs spawned from exposed $F_0$ parents at the 6.5 ppb test level was significantly less than the control in the $F_1$ generation, less than the $F_0$ generation eggs at the 6.5 ppb test level, and less than the $F_1$ generation eggs exposed to 6.5 ppb chlorothalonil but spawned from unexposed $F_0$ parents. This shows that the effect of chlorothalonil on fathead minnows is cumulative and increases as subsequent generations are exposed. In addition, the fact that the parents of $F_1$ eggs had been exposed reduced the hatching success of those eggs, not withstanding the tolerance for chlorothalonil displayed by those $F_0$ parents.
3. According to the one way ANOVA and Duncans Multiple Range test for Variable Response, the growth of fish to exposed chlorothalonil was not reduced significantly compared to the controls.

D. Conclusion

1. Category: Core

2. Rationale: N/A

3. Repairability: N/A
1. Statistical Analysis of the Hatching Success of \( F_0 \) Generation Eggs in Chlorothalonil Compared to the Control and Solvent Control.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

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<tr>
<th>SOURCE</th>
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<th>MEAN SQUARE</th>
<th>F VALUE</th>
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<td>MODEL</td>
<td>6</td>
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<td>CORRECTED TOTAL</td>
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<td>3849.42857143</td>
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<td>0.0027</td>
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R-SQUARE  C.V.  STD DEV  RESPONSE MEAN
0.905960  8.6197  7.19126454  83.42857143

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GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL= .05  DF=7  MS=51.7143

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<td>E 0.6 ppb</td>
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<td>92.500000</td>
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<td>A</td>
<td>90.500000</td>
<td>2</td>
<td>D 1.4 ppb</td>
</tr>
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<td>A</td>
<td>90.000000</td>
<td>2</td>
<td>G control</td>
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<td>C 3.0 ppb</td>
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<td>B 6.5 ppb</td>
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<tr>
<td>A</td>
<td>46.500000</td>
<td>2</td>
<td>A 16.0 ppb</td>
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2. Statistical Analysis of Survival of F₀ Generation Fry at 35 Days in Chlorothalidol as Compared to the Control. The Solvent Control Response was not Included since all fish died in Container "A" of the Solvent Control between Days 16 and 33.

**STATISTICAL ANALYSIS SYSTEM**

13:16 WEDNESDAY, MARCH 26, 1980

**DEPENDENT VARIABLE: RESPONSE**

**SOURCE** | **DF** | **SUM OF SQUARES** | **MEAN SQUARE** | **F VALUE**
---|---|---|---|---
MODEL | 5 | 12746.66666667 | 2549.33333333 | 382.40
ERROR | 6 | 40.00000000 | 6.66666667 | PR > F
CORRECTED TOTAL | 11 | 12786.66666667 |

**R-SQUARE** | **C.V.** | **STD DEV** | **RESPONSE MEAN**
---|---|---|---
0.996672 | 3.1616 | 2.58198890 | 81.66666667

**SOURCE** | **DF** | **TYPE I SS** | **F VALUE** | **PR > F**
---|---|---|---|---
VARIABLE | 5 | 12746.66666667 | 382.40 | 0.0001

**SOURCE** | **DF** | **TYPE IV SS** | **F VALUE** | **PR > F**
---|---|---|---|---
VARIABLE | 5 | 12746.66666667 | 382.40 | 0.0001

**STATISTICAL ANALYSIS SYSTEM**

13:16 WEDNESDAY, MARCH 26, 1980

**GENERAL LINEAR MODELS PROCEDURE**

**DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE**

**MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.**

**ALPHA LEVEL=.05** | **DF=6** | **MS=6.66667**

**GROUPING** | **MEAN** | **N** | **VARIABLE**
---|---|---|---
A | 100.000000 | 2 | D 1.4 ppb
A | 99.000000 | 2 | E 0.6 ppb
A | 94.000000 | 2 | B 6.5 ppb
A | 94.000000 | 2 | C 3.0 ppb
A | 94.000000 | 2 | G control
A | 9.000000 | 2 | A 16.0 ppb
3. Statistical Analysis of Survival of F<sub>0</sub> Generation Fry at 66 Days in Chlorothalolin as Compared to the Control. The Solvent Control Response was not included since all Fish in Container "A" of the Solvent Control died on days 16 and 33.

CHLOROTHALINOL
FATHEAD MINNOW
EGG TO EGG LIFE CYCLE
Daniel Rieder
March 26, 1980

202. 1 STATISTICAL ANALYSIS SYSTEM 2
13:24 WEDNESDAY, MARCH 26, 1980

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE  DF  SUM OF SQUARES  MEAN SQUARE  F VALUE

MODEL  5 13600.0000000000 2720.000000000 123.64
ERROR  6 132.000000000  22.00000000  PR > F
CORRECTED TOTAL  11 13732.000000000 0.0001

R-SQUARE  C.V.  STD DEV  RESPONSE MEAN

0.990387  5.9372  4.69041576  79.00000000

SOURCE  DF  TYPE I SS  F VALUE  PR > F

VARIABLE  5 13600.000000000 123.64 0.0001

SOURCE  DF  TYPE IV SS  F VALUE  PR > F

VARIABLE  5 13600.000000000 123.64 0.0001

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05  DF=6  MS=22

GROUPING  MEAN  N  VARIABLE

A  99.000000  2  E  0.6 ppb
A  96.500000  2  D  1.4 ppb
A  91.500000  2  B  6.5 ppb
A  91.500000  2  C  3.0 ppb
A  91.500000  2  G  control
B  4.000000  2  A  16.0 ppb
4. Statistical Analysis of the Total Spawns of F₀ Generation Fish Treated with Chlorothalindol Compared to the Control and Solvent Control.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE  DF  SUM OF SQUARES  MEAN SQUARE  F VALUE
MODEL  6  1205.57692308  200.92948718  4.22
ERROR  6  285.50000000  47.58333333  PR > F
CORRECTED TOTAL  12  1491.07692308

R-SQUARE  C.V.  STD DEV  RESPONSE MEAN
0.808528  47.1973  6.89806736  14.61538462

SOURCE  DF  TYPE I SS  F VALUE  PR > F
VARIABLE  6  1205.57692308  4.22  0.0516

SOURCE  DF  TYPE IV SS  F VALUE  PR > F
VARIABLE  6  1205.57692308  4.22  0.0516

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05  DF=6  MS=47.5833

GROUPING  MEAN  N  VARIABLE
A  30.500000  2  G  control
B  24.000000  2  D  1.4 ppb
B  16.000000  2  F  solvent control
C  12.500000  2  E  0.6 ppb
B  7.500000  2  B  6.5 ppb
C  4.000000  2  C  3.0 ppb
C  1.000000  1  A  16.0 ppb
5. Statistical Analysis of the Eggs per Spawn of F₀ Generation Fish exposed to Chlorothalinol compared to the Control and Solvent Control.

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE    DF    SUM OF SQUARES    MEAN SQUARE    F VALUE
MODEL      6    15637.73076923    2606.28846154    1.37
ERROR      6    11385.50000000    1897.58333333    PR > F
CORRECTED TOTAL    12    27023.23076923

R-SQUARE    C.V.    STD DEV    RESPONSE MEAN
0.578677    50.3375    43.56125955    86.53846154

SOURCE    DF    ANOVA SS    F VALUE    PR > F
VARIABLE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL = .05  DF = 6  MS = 1897.58

GROUPING    MEAN    N    VARIABLE
A    119.000000    2    E    0.6 ppb
A    113.000000    2    F    solvent control
A    106.500000    2    D    1.4 ppb
A    97.500000    2    G    control
A    87.500000    2    C    3.0 ppb
A    30.000000    2    B    6.5 ppb
A    18.000000    1    A    16.0 ppb
6. Statistical Analysis of the Hatching Success of F1 Generation Eggs in Chlorothalolin Compared to the Control and Solvent Control. Only 18 F1 Eggs were Spawned by F0 parents in the 16.0 ppb Concentration, These were not used to obtain Hatching Data.

| 202. | 1 | STATISTICAL ANALYSIS SYSTEM | 2 |
| 203. | | 13:33 WEDNESDAY, MARCH 26, 1980 |

**GENERAL LINEAR MODELS PROCEDURE**

**DEPENDENT VARIABLE: RESPONSE**

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<td>2126.04545455</td>
<td>1.81</td>
<td>0.2659</td>
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**DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE**

**MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.**

**ALPHA LEVEL=.05**

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<tr>
<td>A</td>
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<td>G</td>
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<td>B</td>
<td>83.000000</td>
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<td>E</td>
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<td>B</td>
<td>79.000000</td>
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<td>D</td>
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<td>B</td>
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<td>C</td>
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<td>B</td>
<td>40.000000</td>
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CHLOROTHALINOL
FATHEAD MINNOW
EGG TO EGG LIFE CYCLE
Daniel Rieder
March 26, 1980

7. Statistical Analysis of Survival of F1 Generation Fry at 34 Days in Chlorothalinol as Compared to the Control and Solvent Control. Only 18 F1 eggs were spawned by F0 parents in the 16.0 ppb test level, therefore no survival data was available for that concentration.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

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R-SQUARE

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ALPHA LEVEL=.05

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<tr>
<td>A</td>
<td>97.500000</td>
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<td>D 1.4 ppb</td>
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<tr>
<td>A</td>
<td>96.500000</td>
<td>2</td>
<td>F solvent control</td>
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<tr>
<td>A</td>
<td>96.000000</td>
<td>2</td>
<td>C 3.0 ppb</td>
</tr>
<tr>
<td>A</td>
<td>91.500000</td>
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<td>E 0.6 ppb</td>
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<td>89.000000</td>
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<td>G control</td>
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<td>80.000000</td>
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<td>B 0.5 ppb</td>
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