081901

Fathead

DATA EVALUATION SHEET

1. CHEMICAL: Bravo 500

2. FORMULATION: Chlorothalonil

3. CITATION

Cary, George A., 1980. A Chronic Study on the Fathead Minnow (Pimephales promelas) with Technical Chlorothalonil. Received 2/19/80. Unpublished report prepared by EG & G, Bionomics for Diamond Shamrock Corporation. (Accession Number 099247)

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 LC_{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 LC_{50} of 23 ppb with 20 to 26 ppb 95% confidence limits. The 35-day LC_{50} and 95% confidence limits were 23 (19-26) ppb, virtually the same as the 96-hour value.

2. All F_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 F_0 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 F_0 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 F_0 generation occurred before day 35.

6. The number of eggs per spawn of F_0 minnows in the 16 and 6.5 ppb concentration was significantly less than in the controls.

7. Second generation (F_1 minnows were apparently more sensitive to chlorothalonil than were first generation (F_0) eggs. The percentage hatching success of F_1 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 F_0 eggs. There was a statistically significant difference between the hatching success of F_0 eggs and F_1 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 statist cal 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_o Generation Eggs in Chlorothalinol Compared to the Control and Solvent Control.

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

1 STATISTICAL ANALYSIS SYSTEM 2
12:51 WEDNESDAY, MARCH 26, 1980

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	6	3487.42857143	581.23809524	11.24
ERROR	7	362.00000000	51.71428571	PR > F
CORRECTED TOTAL	13	3849.42857143		0.0027

R-SQUARE	C.V.	STD DEV	RESPONSE MEAN
0.905960	8.6197	7.19126454	83.42857143

SOURCE	DF	TYPE I SS	F VALUE	PR > F
VARIABLE	6	3487.42857143	11.24	0.0027

SOURCE	DF	TYPE IV SS	F VALUE	PR > F
VARIABLE	6	3487.42857143	11.24	0.0027

1 STATISTICAL ANALYSIS SYSTEM 3
12:51 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=7 MS=51.7143

GROUPING	MEAN	N	VARIABLE	
A	96.000000	2	E	0.6 ppb
A				
A	92.500000	2	F	solvent control
A				
A	90.500000	2	D	1.4 ppb
A				
A	90.000000	2	G	control
A				
A	89.000000	2	C	3.0 ppb
A				
A	79.500000	2	B	6.5 ppb
B	46.500000	2	A	16.0 ppb

6

2. Statistical Analysis of Survival of F_0 Generation Fry at 35 Days in Chlorothalinol 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.

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

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 2
13:16 WEDNESDAY, MARCH 26, 1980

GENERAL LINEAR MODELS PROCEDURE

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		0.0001

R-SQUARE	C.V.	STD DEV	RESPONSE MEAN
0.996872	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

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 3
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				
A	99.000000	2	E	0.6 ppb
A				
A	94.000000	2	B	6.5 ppb
A				
A	94.000000	2	C	3.0 ppb
A				
A	94.000000	2	G	control
B	9.000000	2	A	16.0 ppb

3. Statistical Analysis of Survival of F_0 Generation Fry at 64 Days in Chlorothalinol as Compared to the Control. The Solvent Control Response was not Included since all Fish in Container "A" of the Solvent Control died days 16 and 33.

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1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 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	13600.00000000	2720.00000000	123.64
ERROR	6	132.00000000	22.00000000	PR > F
CORRECTED TOTAL	11	13732.00000000		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	13600.00000000	123.64	0.0001

SOURCE	DF	TYPE IV SS	F VALUE	PR > F
VARIABLE	5	13600.00000000	123.64	0.0001

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 3
13:24 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=22.

GROUPING	MEAN	N	VARIABLE
A	99.000000	2	E 0.6 ppb
A			
A	96.500000	2	D 1.4 ppb
A			
A	91.500000	2	B 6.5 ppb
A			
A	91.500000	2	C 3.0 ppb
A			
A	91.500000	2	G control
B	4.000000	2	A 16.0 ppb

8

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4. Statistical Analysis of the Total Spawns of F_o Generation Fish Treated with Chlorothalinol Compared to the Control and Solvent Control.

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 2
12:59 WEDNESDAY, MARCH 26, 1980

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		0.0516

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

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 3
12:59 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=47.5833

GROUPING	MEAN	N	VARIABLE	
A	30.500000	2	G	control
A				
B A	24.000000	2	D	1.4 ppb
B A				
B A C	16.000000	2	F	solvent control
B C				
B C	12.500000	2	E	0.6 ppb
B C				
B C	7.500000	2	B	6.5 ppb
C				
C	4.000000	2	C	3.0 ppb
C				
C	1.000000	1	A	16.0 ppb

9

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FATHEAD MINNOW
EGG TO EGG LIFE CYCLE
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5. Statistical Analysis of the Eggs per Spawn of F_o Generation Fish exposed to Chlorothalinol compared to the Control and Solvent Control.

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 2
10:16 WEDNESDAY, MARCH 26, 1980

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		0.3549

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	6	15637.73076923	1.37	0.3549

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 3
10:16 WEDNESDAY, MARCH 26, 1980

ANALYSIS OF VARIANCE PROCEDURE

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			
A	113.000000	2	F solvent control
A			
A	106.500000	2	D 1.4 ppb
A			
A	97.500000	2	G control
A			
A	87.500000	2	C 3.0 ppb
A			
A	30.000000	2	B 6.5 ppb
A			
A	18.000000	1	A 16.0 ppb

6. Statistical Analysis of the Hatching Success of F_1 Generation Eggs in Chlorothalinol Compared to the Control and Solvent Control. Only 18 F_1 Eggs were Spawned by F_0 parents in the 16.0 ppb Concentration, These were not used to obtain Hatching Data.

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March 26,1980

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 2
13:33 WEDNESDAY, MARCH 26, 1980

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	5	2126.04545455	425.20909091	1.81
ERROR	5	1176.50000000	235.30000000	PR > F
CORRECTED TOTAL	10	3302.54545455		0.2659

R-SQUARE	C.V.	STD DEV	RESPONSE MEAN
0.643760	20.0159	15.33949152	76.63636364

SOURCE	DF	TYPE I SS	F VALUE	PR > F
VARIABLE	5	2126.04545455	1.81	0.2659

SOURCE	DF	TYPE IV SS	F VALUE	PR > F
VARIABLE	5	2126.04545455	1.81	0.2659

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 3
13:33 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=5 MS=235.3

GROUPING	MEAN	N	VARIABLE	
A	93.000000	2	G	control
A				
B A	83.000000	2	E	0.6 ppb
B A				
B A	79.000000	2	D	1.4 ppb
B A				
B A	78.500000	2	C	3.0 ppb
B A				
B A	68.000000	2	F	solvent control
B				
B	40.000000	1	B	6.5 ppb

11

7. Statistical Analysis of Survival of F_1 Generation Fry at 34 Days in Chlorothalinol as Compared to the Control and Solvent Control. Only 18 F_1 eggs were spawned by F_0 parents in the 16.0 ppb test level, therefore no survival data was available for that concentration.

CHLOROTHALINOL
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1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 2
13:50 WEDNESDAY, MARCH 26, 1980

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	5	288.13636364	57.62727273	3.82
ERROR	5	75.50000000	15.10000000	PR > F
CORRECTED TOTAL	10	363.63636364		0.0840

R-SQUARE	C.V.	STD DEV	RESPONSE MEAN
0.792375	4.1865	3.88587185	92.81818182

SOURCE	DF	TYPE I SS	F VALUE	PR > F
VARIABLE	5	288.13636364	3.82	0.0840

SOURCE	DF	TYPE IV SS	F VALUE	PR > F
VARIABLE	5	288.13636364	3.82	0.0840

1 S T A T I S T I C A L A N A L Y S I S S Y S T E M 3
13:50 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=5 MS=15.1

GROUPING	MEAN	N	VARIABLE	
A	97.500000	2	D	1.4 ppb
A				
A	96.500000	2	F	solvent control
A				
A	96.000000	2	C	3.0 ppb
A				
B A	91.500000	2	E	0.6 ppb
B A				
B A	89.000000	2	G	control
B				
B	80.000000	1	B	6.5 ppb

12