

US EPA ARCHIVE DOCUMENT

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 Unpublished report prepared by EG & G, Bionomics for Diamond Shamrock Corporation.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 IC_{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_{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_{50} of 23 ppb with 20 to 26 ppb 95% confidence limits. The 35-day IC_{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 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 F₀ Generation Eggs in Chlorothalinal Compared to the Control and Solvent Control.

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

202. 1 STATISTICAL ANALYSIS SYSTEM 2
203. 12:51 WEDNESDAY, MARCH 26, 1980

204. GENERAL LINEAR MODELS PROCEDURE

205. DEPENDENT VARIABLE: RESPONSE

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

218. R-SQUARE	C.V.	STD DEV	RESPONSE MEAN
220. 0.905960	8.6197	7.19126454	83.42857143

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

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

231. 1 STATISTICAL ANALYSIS SYSTEM 3
232. 12:51 WEDNESDAY, MARCH 26, 1980

233. GENERAL LINEAR MODELS PROCEDURE

234. DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

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

241. ALPHA LEVEL=.05 DF=7 MS=51.7143

244. GROUPING	MEAN	N	VARIABLE
246. A	96.000000	2	E 0.6 ppb
247. A			
248. A	92.500000	2	F solvent control
249. A			
250. A	90.500000	2	D 1.4 ppb
251. A			
252. A	90.000000	2	G control
253. A			
254. A	89.000000	2	C 3.0 ppb
255. A			
256. A	79.500000	2	B 6.5 ppb
257. A			
258. B	46.500000	2	A 16.0 ppb

6

2. Statistical Analysis of Survival of F₀ Generation Fry at 35 Days in Chlorothalinal 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.

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FATHEAD MINNOW
EGG TO EGG LIFE CYCLE
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202. 1 STATISTICAL ANALYSIS SYSTEM 2
203. 13:16 WEDNESDAY, MARCH 26, 1980

204. GENERAL LINEAR MODELS PROCEDURE

205. DEPENDENT VARIABLE: RESPONSE

209. SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
211. MODEL	5	12746.66666667	2549.33333333	382.40
213. ERROR	6	40.00000000	6.66666667	PR > F
215. CORRECTED TOTAL	11	12786.66666667		0.0001

218. R-SQUARE	C.V.	STD DEV	RESPONSE MEAN
220. 0.996872	3.1616	2.58198890	81.66666667

223. SOURCE	DF	TYPE I SS	F VALUE	PR > F
225. VARIABLE	5	12746.66666667	382.40	0.0001

228. SOURCE	DF	TYPE IV SS	F VALUE	PR > F
230. VARIABLE	5	12746.66666667	382.40	0.0001

231. 1 STATISTICAL ANALYSIS SYSTEM 3
232. 13:16 WEDNESDAY, MARCH 26, 1980

233. GENERAL LINEAR MODELS PROCEDURE

234. DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

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

236. ALPHA LEVEL=.05 DF=6 MS=6.66667

244. GROUPING	MEAN	N	VARIABLE
246. A	100.000000	2	D 1.4 ppb
247. A			
248. A	99.000000	2	E 0.6 ppb
249. A			
250. A	94.000000	2	B 6.5 ppb
251. A			
252. A	94.000000	2	C 3.0 ppb
253. A			
254. A	94.000000	2	G control
255. B			
256. B	9.000000	2	A 16.0 pph

3. Statistical Analysis of Survival of F₀ Generation Fry at 6~~4~~ Days in Chlorothalinalol 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.

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

202. 1 STATISTICAL ANALYSIS SYSTEM 2
203. 13:24 WEDNESDAY, MARCH 26, 1980

204. GENERAL LINEAR MODELS PROCEDURE

205. DEPENDENT VARIABLE: RESPONSE

206.

207. SOURCE	208. DF	209. SUM OF SQUARES	210. MEAN SQUARE	211. F VALUE
212. MODEL	5	13600.0000000	2720.0000000	123.64
213. ERROR	6	132.0000000	22.0000000	PR > F
214. CORRECTED TOTAL	11	13732.0000000		0.0001

215.

216. R-SQUARE	217. C.V.	218. STD DEV	219. RESPONSE MEAN
220. 0.990387	5.9372	4.69041576	79.0000000

221.

222. SOURCE	223. DF	224. TYPE I SS	225. F VALUE	226. PR > F
227. VARIABLE	5	13600.0000000	123.64	0.0001

228.

229. SOURCE	230. DF	231. TYPE IV SS	232. F VALUE	233. PR > F
234. VARIABLE	5	13600.0000000	123.64	0.0001

235. 1 STATISTICAL ANALYSIS SYSTEM 3
236. 13:24 WEDNESDAY, MARCH 26, 1980

237. GENERAL LINEAR MODELS PROCEDURE

238. DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

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

240. ALPHA LEVEL=.05 DF=6 MS=22.

241.

242. GROUPING	243. MEAN	244. N	245. VARIABLE
246. A	99.000000	2	E 0.6 ppb
247. A			
248. A	96.500000	2	D 1.4 ppb
249. A			
250. A	91.500000	2	B 6.5 ppb
251. A			
252. A	91.500000	2	C 3.0 ppb
253. A			
254. A	91.500000	2	G control
255. A			
256. B	4.000000	2	A 16.0 ppb

8

4. Statistical Analysis of the Total Spawns of F₀ Generation Fish Treated with Chlorothalinalol Compared to the Control and Solvent Control.

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FATHEAD MINNOW
EGG TO EGG LIFE CYCLE
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March 26, 1980

202. 1 STATISTICAL ANALYSIS SYSTEM 2
203. 12:59 WEDNESDAY, MARCH 26, 1980

204. GENERAL LINEAR MODELS PROCEDURE

205. 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

231. 1. STATISTICAL ANALYSIS SYSTEM 3
232. 12:59 WEDNESDAY, MARCH 26, 1980

233. GENERAL LINEAR MODELS PROCEDURE

234. DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

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

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

VHD 5
257. 12:59 WEDNESDAY

9

6. Statistical Analysis of the Hatching Success of F₁ Generation Eggs in Chlorothalinol 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.

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

204. GENERAL LINEAR MODELS PROCEDURE

205. DEPENDENT VARIABLE: RESPONSE

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

218. R-SQUARE	C.V.	.STD DEV	RESPONSE MEAN
219. 0.643760	20.0159	15.33949152	76.63636364

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

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

230. 1 STATISTICAL ANALYSIS SYSTEM 3
231. 13:33 WEDNESDAY, MARCH 26, 1980

232. GENERAL LINEAR MODELS PROCEDURE

233. DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

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

235. ALPHA LEVEL=.05 DF=5 MS=235.3

244. GROUPING	MEAN	N	VARIABLE
245. A	93.000000	2	G control
246. A			
247. B A	83.000000	2	E 0.6 ppb
248. B A			
249. B A	79.000000	2	D 1.4 ppb
250. B A			
251. B A	78.500000	2	C 3.0 ppb
252. B A			
253. B A	68.000000	2	F solvent control
254. B			
255. B	40.000000	1	B 6.5 ppb
256. B			

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.

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

1 STATISTICAL ANALYSIS SYSTEM 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 STATISTICAL ANALYSIS SYSTEM 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	96.500000	2	F solvent control
A	96.000000	2	C 3.0 ppb
B	91.500000	2	E 0.6 ppb
B	89.000000	2	G control
B	80.000000	1	B 6.5 ppb