

DATA EVALUATION RECORD
EC₅₀ TEST WITH LEMNA GIBBA
§122-2 (TIER II)

1. **CHEMICAL:** Fentin hydroxide PC Code No.: 083601

2. **TEST MATERIAL:** TPTH Technical Purity: 97.5%

3. **CITATION:**

Author: Hoberg, J.R.

Title: TPTH Technical - Toxicity to Duckweed, *Lemna gibba*

Study Completion Date: November 9, 2000

Laboratory: Springborn Laboratories, Inc.
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Wareham, Massachusetts 02571-1075

Sponsor: TPTH Task Force
c/o Landis International, Inc.
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Valdosta, GA 31603-5126

Laboratory Report ID: 13733.6104

MRID No.: 45276004

DP Barcode: D271849

4. **REVIEWED BY:** Brooke S. Levy, Staff Scientist, Dynamac Corporation

Signature:

Date: 4/17/01

APPROVED BY: Kathleen Ferguson, Ph.D., Senior Staff Scientist, Dynamac Corporation

Signature:

Date: 4/17/01

5. **APPROVED BY:** Tom Steeger, Fishery Biologist, OPP/EFED/ERB IV

Signature:

Date:



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6. STUDY PARAMETERS:**Scientific Name of Test Organism:** *Lemna gibba***Definitive Study Duration:** 7 days**Type of Concentration:** Nominal**7. CONCLUSIONS:**

In this 7-day EC₅₀ test, *Lemna gibba* were exposed to Fenton hydroxide (TPTH technical) at nominal concentrations of 0.073, 0.24, 0.81, 2.7, 9.0, 30, and 100 µg a.i./L (mean measured concentrations: 0.074, 0.24, 0.79, 2.6, 6.6, 24, and 99 µg a.i./L, respectively). Inhibition of frond reproduction increased with increasing test concentrations. The 7-day inhibition rates increasingly ranged from 0.86-85% when compared to the pooled control.

In the mean measured 24 and 99 µg a.i./L treatment groups, observations of small fronds in comparison to the control, less root formation in comparison to the control, slightly chlorotic and chlorotic fronds were generally observed throughout the study duration. By day 7, the number of fronds in the 0.24, 0.79, 2.6, 6.6, 24, and 99 µg a.i./L treatment groups was determined to be significantly reduced when compared to the pooled control. The EC₅₀ value for frond density was 8.3 µg a.i./L; the NOEC was 0.074 µg a.i./L. Inhibition of growth rates increased with increasing test concentrations. Growth rate inhibition rates ranged from 0.27-74% when compared to the pooled control. By day 7, growth rates in the 0.24, 0.79, 2.6, 6.6, 24, and 99 µg a.i./L treatment groups was determined to be significantly reduced when compared to the pooled control. The EC₅₀ value for growth rate was 31 µg a.i./L; the NOEC value was 0.074 µg a.i./L.

This study is classified as SUPPLEMENTAL, due to deviations from the guideline recommendations for an EC₅₀ test with *Lemna gibba* (Subdivision J, §122-2 (TIER II)).

Results Synopsis:**Number of fronds:**EC₅₀: 8.3 µg a.i./L

NOEC: 0.074 µg a.i./L

95% C.I.: 1.9-39 µg a.i./L

Probit Slope: 27

Growth rate:EC₅₀: 31 µg a.i./L

NOEC: 0.074 µg a.i./L

95% C.I.: 5.8-180 µg a.i./L

Probit Slope: 0.96

DP Barcode: D271849

MRID No.: 45276004

8. ADEQUACY OF THE STUDY:**A. Classification:** Supplemental

B. Rationale: The test duration (7 days) is shorter than the guideline recommendation of 14 days. The number of plants and fronds per treatment at test initiation were not reported.

C. Repairability: Future test should be conducted for a period of at least 14 days. The number of plants and fronds used in the study should be provided by the study author to help determine if the data would be useful in a risk assessment.

9. GUIDELINE DEVIATIONS:

1. The test duration (7 days) is shorter than the guideline recommendation of 14 days.
2. The number of plants and fronds were not reported. The guidelines recommend the use of 3-5 plants with 3-5 fronds per plant.

10. SUBMISSION PURPOSE: R (NC)**11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
Species: <i>Lemna gibba</i> G3	<i>Lemna gibba</i>
Number of plants/fronds: 5 plants, 3 fronds per plant	Not reported
Nutrients: Standard formula, e.g. 20X-AAP	Hoagland's medium

B. Test System

Guideline Criteria	Reported Information
Solvent:	Dimethylformamide (DMF)
Temperature: 25°C	Range: 23-26°C (mean min/max.: 23.9/24.3°C)
Light Intensity: 5.0 Klux (±15%)	4.3-6.2 Klux
Photoperiod: Continuous	Continuous
pH: Approximately 5.0	Initial: 5.0 Final: 5.2-5.7
Test System: Static or renewal	Renewal

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	Approximately 3x
Doses: at least 5	Nominal: 0.073, 0.24, 0.81, 2.7, 9.0, 30, and 100 µg a.i./L (mean measured: 0.074, 0.24, 0.79, 2.6, 6.6, 24, and 99 µg a.i./L)
Controls: Negative and/or solvent	Negative and solvent
Replicates per dose: 3 or more	3 replicates
Test duration: 14 days	7 days
Daily observations were made?	Observations of frond counts on days 3, 5, and 7
Method of observations:	Visual

Guideline Criteria	Reported Information
Maximum labeled rate:	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 14 day frond numbers were measured?	Day 3, 5 and 7 frond numbers were measured.
Control frond at 14 days $\geq 2x$ initial count?	Control frond at 7 days $\geq 2x$ day 3 count.
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Replicate data provided.

Dose Response

Mean Measured Concentration (mg a.i./L)	Terminal Average Frond Number	% Inhibition ^a	7-day pH
Control	191	---	5.7
Solvent control	199	---	5.7
Pooled control	195	—	---
0.074	193	0.86	5.6
0.24	175	10	5.6
0.79	156	20	5.7
2.6	156	20	5.6
6.6	103	47	5.5
24	66	66	5.2
99	29	85	5.2

^a Results based on data provided in Table 4 (pp. 24-25) and raw data provided on pages 61-62.

Other significant results: In the 24 and 99 µg a.i./L treatment groups, observations of small fronds in comparison to the control, less root formation in comparison to the control, slightly chlorotic and chlorotic fronds were generally observed throughout the study duration. By day 7, the number of fronds in the 0.24, 0.79, 2.6, 6.6, 24, and 99 µg a.i./L treatment groups was determined to be significantly reduced when compared to the pooled control (William's test). Inhibition of growth rates of *Lemna gibba* increased with increasing test concentrations. In the mean measured 0.074, 0.24, 0.79, 2.6, 6.6, 24, and 99 µg a.i./L treatment groups, growth inhibition rates were 0.27%, 4.3%, 8.9%, 8.6%, 25%, 42%, and 74%, respectively, by day 7. By day 7, growth rates in the 0.24, 0.79, 2.6, 6.6, 24, and 99 µg a.i./L treatment groups were determined to be significantly reduced when compared to the pooled control (William's test).

Statistical results for frond number:

Statistical Method: The control and solvent control groups were compared with a Student's *t*-test. The EC values and 95% confidence intervals were determined by linear regression of response versus mean measured test concentration; the EC values were calculated using 4 linear regression curves. NOEC values were determined with a Williams' test. Normality and homogeneity of variance were determined using Shapiro-Wilks' tests and a Bartlett's tests, respectively. A Williams' test was used to determine the NOEC if the data sets passed the test for normality and homogeneity; a Kruskal-Wallis test was used if the data did not pass the tests for normality and homogeneity.

Number of fronds:

EC₅₀: 8.3 µg a.i./L

NOEC: 0.074 µg a.i./L

95% C.I.: 1.9-39 µg a.i./L

Probit Slope: 27

Growth rate:

EC₅₀: 31 µg a.i./L

NOEC: 0.074 µg a.i./L

95% C.I.: 5.8-180 µg a.i./L

Probit Slope: 0.96

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Data were assessed for normality and homogeneity of variance prior to subsequent analysis. Pooled control data were used to assess treatment effects because two-tailed t-tests revealed no significant differences between negative and solvent control data. Williams' test was used to detect adverse effects of treatment. TOXSTAT software was used for all statistical tests. EC50 estimates were conducted using the method of Bruce and Versteeg via Nuthatch software.

FronD density:EC₅₀: 9.6 µg a.i./L

NOEC: 0.074 µg a.i./L

95% C.I.: 7.6 and 12 µg a.i./L

Probit Slope: 1.02

Biomass:EC₅₀: 32 µg a.i./L

NOEC: 0.074 µg a.i./L

95% C.I.: 28 and 36 µg a.i./L

Probit Slope: 1.22

14. REVIEWER'S COMMENTS:

The reviewer's NOEC and LOEC values were the same as those of the study author. Frond density and growth rate were similarly affected. Both endpoints were significantly reduced at all concentrations except 0.074, the lowest concentration tested. Thus, the NOEC was equal to 0.074 µg a.i./L, the lowest concentration tested. The LOEC equaled 0.24 µg a.i./L. The reviewer estimated slightly higher EC₅₀ values and, so, recommends using the slightly lower estimates of the study author.

The test duration (7 days) is shorter than the guideline recommendation of 14 days.

The number of plants and fronds were not reported. The guidelines recommend the use of 3-5 plants with 3-5 fronds per plant. The study author should provide the number of plants and fronds used in order to determine the usefulness of the data in a risk assessment.

This study was conducted in accordance with USEPA Good Laboratory Practice Regulations and included a Quality Assurance statement.

15. VERIFICATION OF STATISTICAL RESULTS:**FronD production analysis:**

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t-test of Solvent and Blank Controls

Ho: GRP1 MEAN = GRP2 MEAN

 GRP1 (SOLVENT CRTL) MEAN = 190.6667 CALCULATED t VALUE = -1.7489
 GRP2 (BLANK CRTL) MEAN = 199.3333 DEGREES OF FREEDOM = 4
 DIFFERENCE IN MEANS = -8.6667

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	89296.519	12756.646	241.571
Within (Error)	19	1003.333	52.807	
Total	26	90299.852		

Critical F value = 2.54 (0.05,7,19)
 Since F > Critical F REJECT Ho:All groups equal

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WILLIAMS TEST (isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	195.000	195.000	195.000
2	0.074 3	3	193.333	193.333	193.333
3	0.24 3	3	175.000	175.000	175.000
4	0.79 3	3	155.667	155.667	156.000
5	2.6 3	3	156.333	156.333	156.000
6	6.6 3	3	103.000	103.000	103.000
7	24 3	3	65.667	65.667	65.667
8	99 3	3	29.333	29.333	29.333

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISOTONIZED CALC. SIG TABLE DEGREES OF
IDENTIFICATION MEAN WILLIAMS P=.05 WILLIAMS FREEDOM

GRPS 1&2 POOLED	195.000				
0.074	193.333	0.324		1.73	k= 1, v=19
0.24	175.000	3.892	*	1.81	k= 2, v=19
0.79	156.000	7.590	*	1.84	k= 3, v=19
2.6	156.000	7.590	*	1.85	k= 4, v=19
6.6	103.000	17.904	*	1.86	k= 5, v=19
24	65.667	25.170	*	1.87	k= 6, v=19
99	29.333	32.241	*	1.87	k= 7, v=19

s = 7.267

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

Growth rate analysis:

452760-04

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

GRP1 (SOLVENT CRTL) MEAN = 0.3700 CALCULATED t VALUE = -2.0000
GRP2 (BLANK CRTL) MEAN = 0.3767 DEGREES OF FREEDOM = 4
DIFFERENCE IN MEANS = -0.0067

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP ORIGINAL TRANSFORMED ISOTONIZED
IDENTIFICATION N MEAN MEAN MEAN

1	GRPS 1&2 POOLED	6	0.373	0.373	0.373
2	0.074	3	0.370	0.370	0.370
3	0.24	3	0.360	0.360	0.360
4	0.79	3	0.340	0.340	0.340
5	2.6	3	0.340	0.340	0.340
6	6.6	3	0.280	0.280	0.280
7	24	3	0.217	0.217	0.217
8	99	3	0.097	0.097	0.097

452760-04

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WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

ISOTONIZED IDENTIFICATION	CALC. MEAN	SIG. WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
GRPS 1&2 POOLED	0.373				
0.074	0.370	0.497	1.73	k= 1, v=19	
0.24	0.360	1.988 *	1.81	k= 2, v=19	
0.79	0.340	4.969 *	1.84	k= 3, v=19	
2.6	0.340	4.969 *	1.85	k= 4, v=19	
6.6	0.280	13.913 *	1.86	k= 5, v=19	
24	0.217	23.354 *	1.87	k= 6, v=19	
99	0.097	41.193 *	1.87	k= 7, v=19	

s = 0.009

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