

MRID No. 417739-02

DATA EVALUATION RECORD

1. **CHEMICAL:** DPX-T6376.
Shaughnessey No. 122010.
2. **TEST MATERIAL:** DPX-T6376 technical; Batch No. STK 281;
99.2% active ingredient; a white powder.
3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -
Tier 2. Species Tested: Duckweed (*Lemna minor*).
4. **CITATION:** Douglas, M.T. and J.W. Handley. 1988. An
Assessment of the Inhibitory Effect of DPX-T6376 Technical
on the Growth of Duckweed (*Lemna minor*). HRC Report No. DPT
186(b)/881173. Conducted by Huntingdon Research Center
Ltd., Cambridgeshire, UK. Submitted by DuPont de Nemours
(France) S.A., Paris, France. EPA MRID No. 417739-02.
5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: _____
Date: *Charles Lee*
1/21/92
6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: _____
Date: _____

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

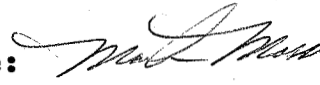
Signature: *Henry T. Craven*
1/21/92
Date: _____
7. **CONCLUSIONS:** This study is scientifically sound but does
not meet the guideline requirements for a Tier 2 aquatic
plant growth and reproduction test. *Lemna gibba* is the
recommended test species for an aquatic macrophyte. Based on
nominal concentrations, the 14-day EC₅₀ was calculated to be
0.36 µg/l with a 95% confidence limit of 0.29-0.43 µg/l.
The NOEC was 0.16 µg/l.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**



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5. **REVIEWED BY:**

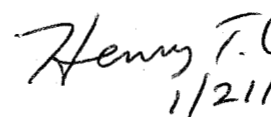
Mark A. Mossler, M.S.
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Date: 1/8/92
6. **APPROVED BY:**

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KBN Engineering and
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Signature: P. Kosalwat
Date: 1/9/92

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

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Date: 1/21/92
7. **CONCLUSIONS:** This study is scientifically sound and meets
the guideline requirements for a Tier 2 aquatic plant growth
and reproduction test. Based on nominal concentrations, the
14-day EC₅₀ was calculated to be 0.36 µg/l with a 95%
confidence limit of 0.29-0.43 µg/l. The NOEC was 0.16 µg/l.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: *Lemna minor* used in the test came from King's College, University of London, London, UK. Stock cultures were maintained in nutrient medium (Appendix 1, attached) under continuous 7,000 lux warm-white illumination, and a temperature of $21 \pm 1^\circ\text{C}$. The culture used as inoculum had been transferred to fresh medium seven days before test initiation.

B. Test System: Test vessels used were 500-ml glass conical flasks covered with transparent lids to prevent evaporation. The test medium was the same as that used for culturing with the pH adjusted to 5.0.

Two-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept in an incubator with conditions identical to those employed in culturing.

C. Dosage: Fourteen-day growth and reproduction test. Five nominal concentrations of 0.04, 0.08, 0.16, 0.32, and $0.64 \mu\text{g/l}$, a solvent control (0.1 ml acetone/l), and a medium control were selected for the definitive test.

Stock solutions were prepared by adding 640 mg of DPX-T6376 technical to 100 ml of acetone and serially diluting accordingly. The test concentrations were prepared by adding $10 \mu\text{l}$ of the appropriate stock to 100 ml of algal medium.

D. Test Design: An inoculum of *Lemna minor* consisted of five plants, each with 2-3 fronds, in each test container (3 containers per treatment). The flasks were renewed with test or control solutions on days 2, 5, 7, 9, and 12. In addition to the 14-day exposure period, the plants were allowed to recover for 7 days in fresh nutrient medium.

Frond counts were made on the days of renewal. Observations of abnormalities were made at this same time and on day 7 of the recovery period. Temperature was recorded daily and pH was recorded immediately prior to renewal of test media.

- E. **Statistics:** The 14-day EC_{50} and associated 95% confidence intervals were computed by fitting the data to a logistic curve. Percent inhibition was calculated based upon the solvent control. The no-observed-effects concentration (NOEC) was estimated using analysis of variance (ANOVA) and Williams' test.
12. **REPORTED RESULTS:** Mean frond count and percent inhibition for each concentration after fourteen days are given in Table 1 (attached). Percent inhibition increased with increasing toxicant concentration. Chlorosis was observed in the highest exposure concentration (0.64 $\mu\text{g/l}$) by day 12 of the test. By day 14, chlorosis and necrosis were evident at this concentration. During the 7-day recovery period, plants in all concentrations demonstrated appreciable frond growth, except for the highest concentration (0.64 $\mu\text{g/l}$).
- The 14-day EC_{50} was calculated to be 0.36 $\mu\text{g/l}$ with a 95% confidence limit of 0.29-0.43 $\mu\text{g/l}$. The NOEC was 0.16 $\mu\text{g/l}$.
- The pH ranged from 5.0 to 5.4 in all test solutions and the controls throughout the test and temperature remained at 21°C.
13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the authors.
- Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160, under the Federal Insecticide, Fungicide, and Rodenticide Act.
14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**
- A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:
- The criteria used to judge frond development was not included in the report.
- The light intensity during the test (7 klux) was higher than recommended (5 klux).
- The recommended test species (*Lemna gibba*) was not used.
- The test temperature (21°C) was lower than recommended (25°C).

B. Statistical Analysis: The reviewer performed probit and ANOVA (Dunnett's) analyses on the 14-day data to determine the EC and NOEC values, respectively. The results obtained by the reviewer are in agreement or are slightly less conservative than those obtained by the authors (see attached printouts).

C. Discussion/Results: Based on nominal concentrations, the 14-day EC₅₀ was calculated to be 0.36 µg/l with a 95% confidence limit of 0.29-0.43 µg/l. The NOEC was 0.16 µg/l.

This study is scientifically sound but does not meet the guideline requirements for a Tier 2 toxicity study using an aquatic macrophyte. Lemna gibba should have been tested.

D. Adequacy of the Study:

- (1) Classification: Supplemental
- (2) Rationale: Refer to Section 14 A.
- (3) Repairability: Not repairable

15. COMPLETION OF ONE-LINER: Yes, 11-27-91.

TABLE 1
Frond counts

Concentration μg/l		No. of fronds							
		Exposure period							Recovery period
Day 0	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	Day 7		
Control	R ₁	14	16	26	33	66	83	129	171
	R ₂	13	14	21	31	47	69	131	187
	R ₃	14	16	24	32	54	93	119	181
	\bar{x}	14	15	24	32	56	82	126	180
Solvent control	R ₁	13	16	23	33	55	79	141	195
	R ₂	13	14	21	33	56	98	130	187
	R ₃	13	14	23	36	50	86	108	166
	\bar{x}	13	15	22	34	54	88	126	183
0.04	R ₁	15	18	25	37	70	131	158	203
	R ₂	13	16	26	36	67	92	121	181
	R ₃	13	16	25	38	64	111	131	172
	\bar{x}	14	17	25	37	67	111	137	185
0.08	R ₁	13	17	24	38	67	101	131	188
	R ₂	14	15	20	30	61	96	120	169
	R ₃	14	16	23	34	59	105	139	190
	\bar{x}	14	16	22	34	62	101	130	182
0.16	R ₁	15	18	24	32	58	94	128	151
	R ₂	14	18	24	32	49	86	123	165
	R ₃	13	16	22	34	55	91	131	168
	\bar{x}	14	17	23	33	54	90	127	161
0.32	R ₁	14	18	21	30	40	66	85	109
	R ₂	14	18	20	30	34	70	84	115
	R ₃	14	18	20	27	42	58	67	97
	\bar{x}	14	18	20	29	39	65	79	107
0.64	R ₁	14	15	17	20	24	28	28	30
	R ₂	14	18	22	25	31	35	39	37
	R ₃	14	16	20	24	26	26	28	27
	\bar{x}	14	16	20	23	27	30	32	31

R₁ - R₃ Replicates 1 - 3

14-day
Inhibition

-9%

-49%

-19%

37%

75%

APPENDIX 1

Nutrient medium

KH_2PO_4	680	mg/l
KNO_3	1515	mg/l
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	1180	mg/l
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	492	mg/l
H_3BO_3	2.86	mg/l
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22	mg/l
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.12	mg/l
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08	mg/l
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	3.62	mg/l
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	5.40	mg/l
Tartaric acid	3.00	mg/l

The pH of this medium after equilibration with air is approximately 5.0.

Lemna frond number

Summary Statistics and ANOVA

Transformation =

None

Group	n	Mean	s.d.	cv%
<i>Concentration (mg/l)</i>				
1 = control	3	126.3333	16.8028	13.3
2 0.04	3	136.6667	19.1398	14.0
3 0.08	3	130.0000	9.5394	7.3
4 0.16	3	127.3333	4.0415	3.2
5*0.32	3	78.6667	10.1160	12.9
6*0.64	3	31.6667	6.3509	20.1

1 = solvent control

NOEC = 0.16 mg/l

Raw data from Table 1 (attached)

*) the mean for this group is significantly less than
the control mean at $\alpha = 0.05$ (1-sided) by Dunnett's test

Minimum detectable difference for Dunnett's test = -24.981475

This difference corresponds to -19.77 percent of control

Between groups sum of squares = 25958.444444 with 5 degrees of freedom.

Error mean square = 149.777778 with 12 degrees of freedom.

Bartlett's test p-value for equality of variances = .421

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

Lemna frond number

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.0400	100	0	0.0000	0.0000	0.0000
0.0800	100	0	0.0000	0.0000	0.0005
0.1600	100	0	0.0000	0.0000	0.0274
0.3200	100	37	0.3700	0.3700	0.2886
0.6400	100	75	0.7500	0.7500	0.7895

Chi - Square Heterogeneity = 7.041

Mu = -0.371652
Sigma = 0.220972

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	6.681900	0.200534	(6.288852,	7.074947)
Slope	4.525467	0.434876	(3.673110,	5.377825)

Theoretical Spontaneous Response Rate = 0.0000

Raw data from Table 1 (attached).

Lemna frond number

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.1301	0.0991	0.1576
EC 5.00	0.1840	0.1508	0.2126
EC10.00	0.2214	0.1880	0.2501
EC15.00	0.2508	0.2178	0.2797
EC50.00	0.4250	0.3883	0.4682
EC85.00	0.7201	0.6329	0.8574
EC90.00	0.8157	0.7060	0.9956
EC95.00	0.9814	0.8286	1.2444
EC99.00	1.3880	1.1155	1.8970

$$y = 6.7 + 4.5(x)$$

$y = \text{probit } \% \text{ inhibition}$
 $x = \log(\text{concentration})$

$$EC_{25} = 0.30$$

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
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14-Day EC₅₀ 99.2 mg/l 95% C.L.
 EC₅₀ = 0.36 pp (0.29-0.43) - probit
 Slope = N/C *
 # plants/vessel = 5 (2-3 standard)

Species: Canth minor Temperature = 21°C

Lab: Huntingdon Research Center McMaster Comp
 MRID # 417739-02 11/23/91

14-Day Dose Level pp 11% Effect
0.04 (0), 0.08 (0), 0.16 (0), 0.32 (37), 0.64 (75)

Comments: NOEC = 0.16 mg/l *
* Based on nominal concentrations
*a- not given

5-Day EC₅₀ _____ 95% C.L.
 EC₅₀ = _____ pp (_____)

Slope = _____ # Cells/ml = _____

Temperature = _____

5-Day Dose Level pp /(% Effect)
 (_____), (_____), (_____), (_____)

Comments: