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:**

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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 EC_{50} 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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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 EC_{50} 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.

6 hrs

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 ±1°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 µg/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 µl 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 µg/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 µg/l).

The 14-day EC_{50} 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.

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 EC_{50} 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

14-day Inhibition

-9%

-4%

-1%

37%

75%

R_1 - R_3 Replicates 1 - 3

APPENDIX 1

Nutrient medium

KH_2PO_4	680	mg/l
KNO_3	1515	mg/l
$Ca(NO_3)_2.4H_2O$	1180	mg/l
$MgSO_4.7H_2O$	492	mg/l
H_3BO_3	2.86	mg/l
$ZnSO_4.7H_2O$	0.22	mg/l
$Na_2MoO_4.2H_2O$	0.12	mg/l
$CuSO_4.5H_2O$	0.08	mg/l
$MnCl_2.4H_2O$	3.62	mg/l
$FeCl_3.6H_2O$	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 (Concentration (mg/l))	n	Mean	s.d.	cv%
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

Minumum 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 = probit % inhibition

x = log (concentration)

$EC_{25} = 0.30$

Shaughnessey # 122010 Chemical Name OPX-T6376 Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day EC_{50}	99.2	EC_{50} = 0.36 ~~pp~~ ug/l (95% C.L.: 0.29-0.43) - probit Slope = N/C ** # plants/vessel = 5 (2-3 fronds each) Species: Lemna minor Temperature = 21°C Lab: Huntingdon Research Center 14-Day Dose Level ~~pp~~ ug/l /(% Effect) 0.04 (0), 0.08 (0), 0.16 (0), 0.32 (37), 0.64 (75) MRID # 417739-02 Comments: NOEC = 0.16 ug/l * * Based on nominal concentrations ** - not given	M. Messler 8/22/91	Core ~~Supplemental~~
5-Day EC_{50}	_____	EC_{50} = pp (95% C.L.: ( )) Slope = # Cells/ml = Species: Temperature = Lab: 5-Day Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) MRID # Comments:	_____	_____