(3-16-92)

Addendum

14D. Adequacy of Study: This study should be changed from core to supplemental because of the following deviations from the SEP and ASTM (1988): 1) On page 11 of the report, the author states "there was no statistically significant difference between the test group and the solvent control..." However, the report does not document the use of a solvent control. 2) The dilution water was unfiltered fish culture water with no analysis of ammonia. These discrepancies were noted by KBN on p. 4 of their report.

This study appears to be scientifically sound but will not fulfill requirements for registration. The study may be repairable if information on the solvent control and the dilution water is submitted to EEB.

Miachel Rexrode 3/5/92

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DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorsulfuron.
Shaughnessey No. 118601.

2. **TEST MATERIAL:** DPX-W4189-94 (H-17,453); Benzenesulfonamide, 2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]carbonyl]-; CAS No. 64902-72-3; 95.4% active ingredient; a tan solid.

3. **STUDY TYPE:** *Daphnia magna* Life-Cycle (21-Day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Hutton, D.G. 1989. Chronic Toxicity of DPX-W4189-94 to *Daphnia magna*. Laboratory Report No. 87-89. Prepared by E.I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE. Submitted by E.I. du Pont de Nemours and Co., Inc., Wilmington, DE. EPA MRID No. 419764-04 for summary report and EPA MRID No. 419764-08 for data addendum.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 2/12/92

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M. Rifici
Date: 2/12/92

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

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a chronic, static-renewal toxicity test for the freshwater invertebrate, *Daphnia magna*. The MATC for DPX-W4189-94, based on the most sensitive biological parameter, daphnid reproduction, was >20 and <36 mg/l mean measured concentrations (geometric mean MATC = 26.8 mg/l).

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: *Daphnia magna* (<24 hours old) were obtained from in-house cultures. The cultures were housed in 2-l glass beakers containing 1.8 l of filtered fish tank water and held at approximately 20°C. Neonates were collected from 14-day old adults for use in the test. The adult daphnids were fed a trout chow and yeast diet.

B. Test System: The test vessels were 250-ml glass beakers containing 200 ml of test solution at a depth of 6.8 cm. The test solutions were renewed on days 3, 5, 7, 10, 12, 14, 17, and 19. The test solutions were held at approximately 20°C. The photoperiod was 16-hour light/8-hour dark with a light intensity of approximately 550 lux.

The dilution water was aerated, filtered well water which had been distributed to fathead minnow cultures then filtered (0.8 µm) before use in the test. At the time of test initiation, the dilution water had a pH of 7.4, a conductivity of 165 µmhos/cm, and a hardness and alkalinity of 72 and 76 mg/l as $CaCO_3$, respectively.

A primary stock solution was prepared by diluting 2 g of test substance to a volume of 2 l with dilution water. "Dissolving of the test material will be aided by 1N NaOH (E.M. Science Lot #80/91) to pH<9." Test solutions were prepared 2-3 days prior to the day of renewal.

C. Dosage: Twenty-one-day, static-renewal, life-cycle chronic toxicity test. Six nominal concentrations (9, 19, 38, 75, 150, and 300 mg/l) were selected for the test. A dilution water control was also included.

D. Design: Each treatment level consisted of 7 replicates containing one daphnid each (for monitoring survival, growth, and reproduction) and 3 replicates containing five daphnids each (for monitoring survival only). Daphnids were added to the test beakers by "randomly picking them from a single transfer tank." The daphnids were transferred to fresh solutions containing food (30 mg/l) at each renewal. The time of the last feeding was the morning of test day 19.

Survival and reproduction of the daphnids were determined at each renewal and at test termination. The length of all daphnids was determined at test termination.

The dissolved oxygen concentration and pH of new and old solutions of the control, low, middle, and high concentrations were measured on days 0, 3, 5, 7, 10, 12, 14, 17, 19, and 21. The temperature of the control was measured daily. Test temperature was also continuously monitored using a recording thermometer. The conductivity, hardness, and alkalinity of the dilution water control were measured at test initiation and weekly thereafter.

Samples of the new test solutions were taken on days 0, 7, and 14, and samples of the old test solutions were taken on days 7, 14, and 21 for quantitative analysis of DPX-W4189-94 by high liquid chromatography (HPLC).

E. **Statistics:** Reproduction (the total number of young produced and the number of young produced per female reproductive day) and growth (length) were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. All conclusions of statistical significance were based on $p \leq 0.05$.

12. **REPORTED RESULTS:** The mean measured concentrations were 9.3, 20, 36, 79, 140, and 300 mg/l which represent 92.3-105.3% of nominal concentrations (Table 6, attached).

The concentration of the test material had no significant effect on survival since survival for all test levels and the control ranged from 95 to 100%. Length was only significantly reduced at the highest concentration (300 mg/l). Reproduction (total number of young produced and the number of young produced per reproductive day) was significantly affected at $\geq$36 mg/l mean measured concentration (Table 2, attached).

The pH of the test solutions ranged from 7.2 to 8.5. Dissolved oxygen ranged from 3.2 to 8.8 mg/l. The temperature of the control solution was 19.6-21.0°C during the study. The alkalinity, hardness, and conductivity of the dilution water were 76-91 mg/l as $CaCO_3$, 72-88 mg/l as $CaCO_3$, and 160-165 μmhos/cm, respectively, during the exposure.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

"The NOEL was 20 mg/liter measured concentration. The MATC was between 20 and 36 mg/l measured concentration."

A Good Laboratory Practice Statement, signed by the study director and a company representative, was included in the report indicating that this study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations (40 CFR 160). The report also included Quality Assurance Documentation which was signed by a quality assurance auditor.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the SEP and ASTM (1988), but deviated as follows:

The highest measured concentration at 156.4 mg/l mean measured concentration was more than twice the lowest measured concentration.

Treatments must be randomly assigned to the test chambers. The report does not mention if the treatments were randomly assigned.

On page 11 of the report, the author states "there was no statistically significant differences between the test group and the solvent control group for survival..." No solvent control was used in this study. This is a discrepancy in the report.

The age of the adult daphnids which produced the test organisms were younger (14 days) than recommended (21 days) by the guidelines.

Daphnid length was measured to the nearest 0.1 mm; the SEP recommends measurement to the nearest 0.01 mm.

The report does not indicate whether test chambers were covered as recommended to prevent evaporation and to keep out extraneous contamination.

The hardness of the dilution water (76-88 mg/l as $CaCO_3$) was lower than recommended (160-180 mg/l as $CaCO_3$) in the SEP. The range of hardness of the dilution was greater than 10% of the average.

The dilution water was well water which had passed through a fish culture unit. No justification was given why a fish culture water was used. The ammonia

content may have been elevated and should have been checked prior to use.

The report does not indicate whether the dilution water was intensively aerated prior to addition of the test material.

Temperature was measured in only in the control. ASTM guidelines require that temperature be measured concurrently in all test chambers near the beginning, middle, and end of the study.

Light to dark and dark to light transition periods were not used as recommended in the guidelines.

B. **Statistical Analysis:** The reviewer used one-way ANOVA and Dunnett's test (Toxstat Version 3.3) to analyze the reproduction (the number of young produced per female reproductive day) and length of daphnids. The results of the analyses were the same as those of the author (printouts, attached).

C. **Discussion/Results:** The deviations listed above probably did not alter the results of this study. Although, the highest measured concentration at 156.4 mg/l mean measured concentration was more than twice the lowest measured concentration, the MATC limits were not affected by this test level. This study is scientifically sound and meets the guideline requirements for a chronic, static-renewal toxicity test for the freshwater invertebrate, *Daphnia magna*. The MATC of DPX-W4189-94, based on the most sensitive biological parameter, daphnid reproduction, was >20 and <36 mg/l mean measured concentrations (geometric mean = 26.8 mg/l).

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, January 27, 1992.

REFERENCES: ASTM. 1988. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. E 1193-87.

TABLE 6

MEASURED CONCENTRATIONS OF DPX-W4189-94 IN TEST SOLUTIONS
(MR 4581-655)

Nominal Test Concentration mg/L	Measured Test Concentration, mg/L							
	Day 0 Fresh	Day 7 Old	Day 7 Fresh	Day 14 Old	Day 14 Fresh	Day 21 Fresh	Average*	REVIEWER'S AVERAGE
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
9	6.3	8.6	8.8	10.9	11.0	10.1	9.3	9.3
19	17.8	19.6	19.3	19.7	22.4	21.5	20	20
38	40.7	33.6	39.8	29.8	38.0	35.9	36	36
75	89.1	67.8	82.0	70.9	86.4	78.4	79	79
150	133.3	139.3	151.9	74.5	157.1	156.4	140	135
300	297.5	322.6	253.3	307.9	297.1	307.6	300	298

* Average values are reported to two significant figures.

TABLE 2

CHRONIC EFFECTS OF DPX-W4189-94 (H-17,453) ON Daphnia magna
(MR 4581-655)

Nominal Test Concen. (mg/L)	Measured Test Concen. (mg/L)	Mean Values				
		Mortality Percent Survival	Reproduction			Length
			First Day	No. of Young	Young Per Day	(mm)
Control	0.0	100	10.0	195	16.2	4.5
9	9.3	100	9.6	176	14.3	4.4
19	20	100	10.0	175	14.6	4.3
38	36	100	10.0	159#	13.2#	4.3
75	79	100	10.0	151#	12.6#	4.4
150	140	95	10.3	98#	8.4#	4.1
300	300	100	10.0	58#	4.8#	4.1#

Significantly different ($p < 0.05$) from Water Control group by Dunnett's Test.

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TITLE: DPX-W4189-94: Length of Exposed Daphnia magna
FILE: b:41976404.len
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	6.7000	6.7000
1	Control	2	6.8000	6.8000
1	Control	3	6.7000	6.7000
1	Control	4	6.9000	6.9000
1	Control	5	6.8000	6.8000
1	Control	6	7.1000	7.1000
1	Control	7	7.2000	7.2000
2	9 mg/l	1	6.9000	6.9000
2	9 mg/l	2	6.7000	6.7000
2	9 mg/l	3	7.0000	7.0000
2	9 mg/l	4	7.0000	7.0000
2	9 mg/l	5	6.9000	6.9000
2	9 mg/l	6	6.3000	6.3000
2	9 mg/l	7	6.3000	6.3000
3	19 mg/l	1	6.5000	6.5000
3	19 mg/l	2	6.9000	6.9000
3	19 mg/l	3	6.8000	6.8000
3	19 mg/l	4	7.3000	7.3000
3	19 mg/l	5	6.5000	6.5000
3	19 mg/l	6	6.0000	6.0000
3	19 mg/l	7	6.1000	6.1000
4	38 mg/l	1	7.0000	7.0000
4	38 mg/l	2	6.9000	6.9000
4	38 mg/l	3	7.0000	7.0000
4	38 mg/l	4	5.3000	5.3000
4	38 mg/l	5	6.8000	6.8000
4	38 mg/l	6	6.3000	6.3000
4	38 mg/l	7	6.6000	6.6000
5	75 mg/l	1	6.8000	6.8000
5	75 mg/l	2	7.1000	7.1000
5	75 mg/l	3	6.9000	6.9000
5	75 mg/l	4	6.9000	6.9000
5	75 mg/l	5	7.2000	7.2000
5	75 mg/l	6	6.2000	6.2000
5	75 mg/l	7	6.7000	6.7000
6	150 mg/l	1	6.9000	6.9000
6	150 mg/l	2	6.6000	6.6000
6	150 mg/l	3	6.7000	6.7000
6	150 mg/l	4	6.1000	6.1000
6	150 mg/l	5	5.6000	5.6000
6	150 mg/l	6	6.8000	6.8000
6	150 mg/l	7	5.9000	5.9000
7	300 mg/l	1	6.1000	6.1000
7	300 mg/l	2	6.0000	6.0000
7	300 mg/l	3	6.4000	6.4000
7	300 mg/l	4	6.0000	6.0000
7	300 mg/l	5	6.5000	6.5000
7	300 mg/l	6	6.7000	6.7000
7	300 mg/l	7	6.6000	6.6000

DPX-W4189-94: Length of Exposed Daphnia magna
File: b:41976404.len Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1.941	0.324	1.960
Within (Error)	42	6.931	0.165	
Total	48	8.873		

Critical F value = 2.34 (0.05,6,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DPX-W4189-94: Length of Exposed Daphnia magna
File: b:41976404.len Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	6.886	6.886		
2	9 mg/l	6.729	6.729	0.724	
3	19 mg/l	6.586	6.586	1.382	
4	38 mg/l	6.557	6.557	1.513	
5	75 mg/l	6.829	6.829	0.263	
6	150 mg/l	6.371	6.371	2.368	
7	300 mg/l	6.329	6.329	2.566	*

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

DPX-W4189-94: Length of Exposed Daphnia magna
File: b:41976404.len Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	7			
2	9 mg/l	7	0.515	7.5	0.157
3	19 mg/l	7	0.515	7.5	0.300
4	38 mg/l	7	0.515	7.5	0.329
5	75 mg/l	7	0.515	7.5	0.057
6	150 mg/l	7	0.515	7.5	0.514
7	300 mg/l	7	0.515	7.5	0.557

TITLE: DPX-W4189-94: Young Produced/Female Reproductive Day
FILE: b:41976404.frd
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	15.4000	15.4000
1	Control	2	16.7000	16.7000
1	Control	3	17.3000	17.3000
1	Control	4	17.0000	17.0000
1	Control	5	14.4000	14.4000
1	Control	6	17.4000	17.4000
1	Control	7	15.5000	15.5000
2	9 mg/l	1	14.3000	14.3000
2	9 mg/l	2	17.1000	17.1000
2	9 mg/l	3	11.7000	11.7000
2	9 mg/l	4	13.0000	13.0000
2	9 mg/l	5	15.8000	15.8000
2	9 mg/l	6	12.3000	12.3000
2	9 mg/l	7	15.8000	15.8000
3	19 mg/l	1	13.1000	13.1000
3	19 mg/l	2	14.8000	14.8000
3	19 mg/l	3	17.1000	17.1000
3	19 mg/l	4	16.8000	16.8000
3	19 mg/l	5	11.6000	11.6000
3	19 mg/l	6	12.4000	12.4000
3	19 mg/l	7	16.2000	16.2000
4	38 mg/l	1	11.8000	11.8000
4	38 mg/l	2	12.8000	12.8000
4	38 mg/l	3	16.2000	16.2000
4	38 mg/l	4	13.7000	13.7000
4	38 mg/l	5	12.5000	12.5000
4	38 mg/l	6	13.9000	13.9000
4	38 mg/l	7	11.6000	11.6000
5	75 mg/l	1	14.2000	14.2000
5	75 mg/l	2	12.2000	12.2000
5	75 mg/l	3	14.9000	14.9000
5	75 mg/l	4	10.9000	10.9000
5	75 mg/l	5	12.4000	12.4000
5	75 mg/l	6	12.5000	12.5000
5	75 mg/l	7	11.0000	11.0000
6	150 mg/l	1	6.5000	6.5000
6	150 mg/l	2	7.1000	7.1000
6	150 mg/l	3	7.7000	7.7000
6	150 mg/l	4	10.8000	10.8000
6	150 mg/l	5	9.0000	9.0000
6	150 mg/l	6	10.8000	10.8000
6	150 mg/l	7	6.7000	6.7000
7	300 mg/l	1	5.0000	5.0000
7	300 mg/l	2	3.8000	3.8000
7	300 mg/l	3	6.2000	6.2000
7	300 mg/l	4	4.4000	4.4000
7	300 mg/l	5	6.8000	6.8000
7	300 mg/l	6	4.5000	4.5000
7	300 mg/l	7	2.9000	2.9000

DPX-W4189-94: Young Produced/Female Reproductive Day
File: b:41976404.frd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	676.633	112.772	38.731
Within (Error)	42	122.291	2.912	
Total	48	798.925		

Critical F value = 2.34 (0.05,6,40)
Since F > Critical F REJECT Ho:All groups equal

DPX-W4189-94: Young Produced/Female Reproductive Day
File: b:41976404.frd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	16.243	16.243		
2	9 mg/l	14.286	14.286	2.146	
3	19 mg/l	14.571	14.571	1.833	
4	38 mg/l	13.214	13.214	3.320	*
5	75 mg/l	12.586	12.586	4.010	*
6	150 mg/l	8.371	8.371	8.630	*
7	300 mg/l	4.800	4.800	12.546	*

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

DPX-W4189-94: Young Produced/Female Reproductive Day
File: b:41976404.frd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	7			
2	9 mg/l	7	2.162	13.3	1.957
3	19 mg/l	7	2.162	13.3	1.671
4	38 mg/l	7	2.162	13.3	3.029
5	75 mg/l	7	2.162	13.3	3.657
6	150 mg/l	7	2.162	13.3	7.871
7	300 mg/l	7	2.162	13.3	11.443