1/5/94 (PM)
2-2-94

MRID No. 416138-06

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin (or Benfluralin).
Shaughnessey Number: 084301.

2. **TEST MATERIAL:** Benefin (Compound 054521); N-(n-butyl)-N-ethyl-2,6-dinitro-α,α,α-trifluoro-ρ-toluidine; Lot No. 231EF4; 95.88% active ingredient; 0.1 mg/l aqueous solubility.

72-4(b)

3. **STUDY TYPE:** Freshwater Invertebrate Flow-Through Life-Cycle Chronic Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Mohr, R.R., P.J. Cocke, and G.R. Koenig. 1990. The Chronic Toxicity of Benefin to *Daphnia magna* in a Flow-Through Life-Cycle Test. Study No. C01090. Performed by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco. EPA MRID No. 416138-06.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 7/2/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 7/2/92

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

Signature: 2/2/94 2-2-94
Date:

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the requirements for a chronic, flow-through toxicity test using the freshwater invertebrate, *Daphnia magna*. The design of the test vessels was unusual and may have allowed neonate daphnids to escape, leading to inaccurate reproduction data. Under the conditions of the study, the MATC was >15.5 and <30.8 µg/l mean measured concentrations, based on significantly affected reproduction. The geometric mean MATC was 21.8 µg/l mean measured concentration.

8. **RECOMMENDATIONS:** See Section 14.D.(3).

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** *Daphnia magna* (≤24 hours old) used in this test were obtained from in-house cultures. The daphnid cultures were maintained for 14 days at 18.2-20.8°C in conditioned well water with a total hardness of 103 to 137 mg/l as $CaCO_3$, a total alkalinity of 110 to 145 mg/l as $CaCO_3$, and a conductivity of 210 to 255 µS/cm. Daphnids in the cultures were fed green algae (*Selenastrum capricornutum*) and cerophyl daily.

B. **Test System:** A proportional diluter system was used to mix and deliver test solutions to four replicate test beakers per treatment level. Each 200-ml glass test beaker was fitted with a rubber stopper that held a delivery tube and an overflow tube to eliminate the air/water interface.

The flow rate from the diluter provided approximately 10 volume replacements per day. The diluter cycled every 90 minutes. The test chambers were arranged in a randomized-block design. The photoperiod provided 16 hours of light (2-4 $\mu E/m^2/sec$) and 8 hours of darkness.

An automatic feeding system mixed a suspension of green algae (*Selenastrum capricornutum*) with the test solutions at a concentration of approximately 150,000 cells/ml on days 0-7, 300,000 cells/ml on days 8-13, and 500,000 cells/ml on days 14-21.

The dilution water was conditioned well water with a total hardness of 103 to 120 mg/l as $CaCO_3$, a total alkalinity of 130 to 134 mg/l as $CaCO_3$, and a specific conductivity of 279 to 360 µmhos/cm during testing.

A primary stock solution was prepared weekly by dissolving 100 mg of benefin in acetone to a total volume of 50 ml. A diluter stock solution (200 µg/l) was prepared daily by adding 4.0 ml of the primary stock solution to 40 l of dilution water to provide the highest nominal concentration. This solution was proportionally diluted (50% dilution factor) to provide the remaining four concentrations. An injector was used to automatically deliver acetone to the solvent control at a concentration of 0.1 ml/l, which was equal

to the highest acetone concentration in any treatment level.

C. **Dosage:** Twenty-one-day, flow-through toxicity test. Five nominal concentrations (18.5, 25, 50, 100, and 200 µg/l) were tested. A dilution water control and solvent control (0.1 ml/l) were also included.

D. **Design:** Five daphnids were impartially selected and randomly added to each of four replicate chambers per concentration and control (20 daphnids per treatment level). Survival, abnormal effects, and reproduction of the adults were monitored three times per week (Monday, Wednesday, and Friday). The day of first brood production was noted for each replicate. The length of all daphnids was measured to the nearest 0.01 mm at test termination.

The dissolved oxygen concentration, temperature, and pH of the solutions in each vessel were measured at test initiation and termination. The above parameters were measured in one replicate of each level twice weekly. The temperature of the water discharged from the test vessels was monitored continuously using a probe and recorder. The total hardness, total alkalinity, and conductivity of the dilution water control and highest test concentration were measured weekly.

Composite water samples were collected on days 0, 14, and 21. On day 6, a sample was taken from each treatment replicate. The samples were analyzed using gas chromatography.

E. **Statistics:** Survival data were arcsine square root transformed prior to analysis. A one-tailed Dunnett's t-test was used to compare survival in the treatments to that of the solvent control. Body length and the total number of young produced per female were analyzed using one-tailed Dunnett's t-tests. All conclusions of statistical significance were based on $p \leq 0.05$.

12. **REPORTED RESULTS:** The mean measured concentrations were 9.6, 15.5, 30.8, 64.9, and 135.7 µg/l (Table 1, attached). Measured concentrations represented 52 to 68% of nominal concentrations. No benefin was detected in the control solutions.

No significant reduction in survival relative to the solvent control occurred at any benefin concentration. Mean survival percentages by concentration ranged from 90 to

100%. Survival in the dilution water control and solvent control were 70 and 100%, respectively. No treatment-related sublethal effects were observed.

Reproduction (number of young produced per female) was significantly reduced at concentrations ≥30.8 µg/l. Adult daphnid length was significantly affected at 135.7 µg/l

During the test, the pH ranged from 7.9 to 8.8, the dissolved oxygen concentration was 7.9-9.4 mg/l (≥85% of saturation), and the temperature averaged 19.5 ±0.4°C. The hardness, alkalinity, and conductivity of the dilution water control and high test concentration were 103-120 mg/l as $CaCO_3$, 130-135 mg/l as $CaCO_3$, and 277-360 µS/cm, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on the results from this study, the highest chronic no-observed-effect concentration (NOEC) of benefin for *Daphnia magna* was 15.5 µg/l."

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:** At the present time, no SEP exists for flow-through, life-cycle chronic toxicity tests with daphnids. A proposed standard practice for conducting *Daphnia magna* flow-through chronic toxicity tests (ASTM, 1985) was used to judge the soundness of the study. The test procedures were generally in accordance with ASTM (1985), except for the following:

The recommended test design was 4 replicates containing 10 daphnids each for a total of 40 daphnids per concentration. The design used in this test was 4 replicates containing only 5 daphnids each for a total of 20 per concentration.

The physical state of the test material was not reported.

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

B. **Statistical Analysis:** The reviewer calculated the number of young produced per reproductive day from raw data provided in the report (pp. 56-63). The data were analyzed using one-way analysis of variance (ANOVA) and Williams' test (printout 1, attached). Reproduction at concentrations ≥30.8 µg/l were significantly lower than solvent control reproduction. Adult daphnid length was analyzed using two-way ANOVA and Bonferroni's test (Crunch Version 3). Only the daphnids at 135.7 µg/l were significantly smaller than solvent control daphnids (printouts 2 and 3, attached).

C. **Discussion/Results:** The test vessels used in this study (stoppered 200-ml glass beakers) were unusual. The benefits of using these vessels was unclear to the reviewer. If the test material was highly volatile, the stoppered beakers may help eliminate cross contamination. However, since the air/water interface was eliminated, neonate daphnids may have escaped through the outflow during solution addition. During each cycle, 125 ml of solution were added to each beaker. The diluter cycled 16 times per day. The authors describe the stopper as having a delivery tube and an overflow tube. There was no mention of a physical barrier (i.e., nylon screening) at the intake of the outflow tube. If neonates were lost, it is likely that beakers containing higher numbers of neonates (presumably control beakers) lost proportionally more. Therefore, the differences between control and treatment reproduction would be artificially smaller and the maximum-acceptable-toxicant concentration (MATC) less conservative. The authors need to discuss any measures used to prevent the loss of neonates from the test chambers.

This study is scientifically sound but does not meet the requirements for a chronic, flow-through toxicity test using the freshwater invertebrate, *Daphnia magna*. The design of the test vessels was unusual and may have allowed neonate daphnids to escape leading to inaccurate reproduction data. Under the conditions of the study, the MATC, based on the most sensitive parameter, reproduction, was >15.5 and <30.8 µg/l mean measured concentrations. The geometric mean MATC was 21.8 µg/l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The design of the test vessels was unusual and may have allowed neonate daphnids to escape leading to inaccurate reproduction data.

(3) **Repairability:** The authors must submit evidence that no neonates were lost from the test vessels.

15. **COMPLETION OF ONE-LINER:** Yes, 6-26-92.

Printout 1

416138-06, BENEFIN, YOUNG/REPRODUCTIVE DAY
File: A:41613806.DT1 Transform: NO TRANSFORMATION

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

GRP1 (SOLVENT CRTL) MEAN =	6.6800	CALCULATED t VALUE =	0.6496
GRP2 (BLANK CRTL) MEAN =	6.2100	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.4700		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	22.696	3.783	7.347
Within (Error)	21	10.812	0.515	
Total	27	33.509		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	4	6.680	6.680	6.680
2	DILUT. CONTROL	4	6.210	6.210	6.573
3	9.6	4	6.800	6.800	6.573
4	15.5	4	6.708	6.708	6.573
5	30.8	4	5.650	5.650	5.676
6	64.9	4	5.702	5.702	5.676
7	135.7	4	4.045	4.045	4.045

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	6.680				
DILUT. CONTROL	6.573	0.212		1.72	k= 1, v=21
9.6	6.573	0.212		1.80	k= 2, v=21
15.5	6.573	0.212		1.83	k= 3, v=21
30.8	5.676	1.978	*	1.84	k= 4, v=21
64.9	5.676	1.978	*	1.85	k= 5, v=21
135.7	4.045	5.193	*	1.85	k= 6, v=21

Printout 2

Analysis of Variance File: BENDAPLN Date: 05-26-1992

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors: C	R	Mean measured concentration (ug/l)	N	Mean	S.D.
*	*		131	3.9179	0.1916
1	*	Solvent Control	20	3.9570	0.2342
2	*	Control	15	3.8987	0.1613
3	*	9.6	19	4.0421	0.1705
4	*	15.5	18	4.0144	0.1956
5	*	30.8	20	3.8920	0.1543
6	*	64.9	19	3.8642	0.1443
# 7	*	135.7	20	3.7650	0.1322
*	1		32	3.9012	0.2402
*	2		33	3.9509	0.1803
*	3		34	3.9088	0.1872
*	4		32	3.9100	0.1541
1	1		5	3.7960	0.3928
1	2		5	3.9600	0.1881
1	3		5	4.0720	0.1026
1	4		5	4.0000	0.0949
2	1		3	3.8800	0.1400
2	2		5	4.0160	0.2012
2	3		4	3.8450	0.1012
2	4		3	3.7933	0.0945
3	1		4	4.1200	0.1633
3	2		5	4.1000	0.1679
3	3		5	3.9000	0.1995
3	4		5	4.0640	0.0817
4	1		5	4.0760	0.1584
4	2		4	3.9850	0.1879
4	3		5	3.9400	0.2760
4	4		4	4.0600	0.1641
5	1		5	3.8080	0.2072
5	2		5	3.8840	0.1410
5	3		5	4.0040	0.1014
5	4		5	3.8720	0.1221
6	1		5	3.9200	0.1876
6	2		4	3.9450	0.0900
6	3		5	3.7800	0.1649
6	4		5	3.8280	0.0657
7	1		5	3.7440	0.1322
7	2		5	3.7720	0.1534
7	3		5	3.8080	0.1847
7	4		5	3.7360	0.0623

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

ax for testing homogeneity of between subjects variances: 39.76

mber of variances= 28 df per variance= 4.

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

alysis of Variance Dependent variable: LENGTH

urce	df	SS (H)	MSS	F	P
tween Subjects	130	4.7714			
C (CONC)	6	1.0328	0.1721	5.878	0.0000
R (REP)	3	0.0496	0.0165	0.564	0.6429
CR	18	0.6729	0.0374	1.277	0.2173
Subj w Groups	103	3.0162	0.0293		

Printout 3

Analysis of Variance File: BENDAPLN Date: 05-26-1992

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	3.957	6	3.864
2	3.899	7	3.765
3	4.042		
4	4.014		
5	3.892		

Comparison	Tukey-A*	Bon-ferroni	Dunnett
1 > 2			
1 < 3			
1 < 4			
1 > 5			
1 > 6			
1 > 7	0.0500	0.0126	0.0100
2 < 3			N.A.
2 < 4			N.A.
2 > 5			N.A.
2 > 6			N.A.
2 > 7			N.A.
3 > 4			N.A.
3 > 5			N.A.
3 > 6	0.0500	0.0382	N.A.
3 > 7	0.0100	0.0000	N.A.
4 > 5			N.A.
4 > 6			N.A.
4 > 7	0.0100	0.0005	N.A.
5 > 6			N.A.
5 > 7			N.A.
6 > 7			N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500). A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Shaughnessey # 084301 Chemical Name Benefin (Benfluralin) Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Chronic Fish		Concentrations Tested (pp___) -		
Species:		MATC - >_____<_____ pp___.		
Lab:		Effected Parameters -		
MRID #		Control Mortality (%) - Solvent Control Mortality (%) -		
		Comments:		
Chronic Invertebrate	95.88	Concentrations Tested (pp b*) - 9.6, 15.5, 30.8, 64.9, 135.7		
Species: Daphnia magna		MATC - > 15.5 < 30.8 pp b*.	LMR 6/26/92	Supplemental
Lab: Lilly Research Labs.		Effected Parameters - Reproduction (young per adult; young/Reproductive days), length		
MRID # 416138-06		Control Mortality (%) - 30 Solvent Control Mortality (%) - 0		
		Comments: * mean measured concentrations		

TITLE: 416138-06, BENEFIN, YOUNG/REPRODUCTIVE DAY
FILE: A:41613806.DT1
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	6.1200	6.1200
1	SOLVENT CONTROL	2	5.9700	5.9700
1	SOLVENT CONTROL	3	7.1500	7.1500
1	SOLVENT CONTROL	4	7.4800	7.4800
2	DILUT. CONTROL	1	7.7500	7.7500
2	DILUT. CONTROL	2	4.9700	4.9700
2	DILUT. CONTROL	3	6.6300	6.6300
2	DILUT. CONTROL	4	5.4900	5.4900
3	9.6	1	7.2000	7.2000
3	9.6	2	6.9800	6.9800
3	9.6	3	5.9200	5.9200
3	9.6	4	7.1000	7.1000
4	15.5	1	6.4500	6.4500
4	15.5	2	6.1300	6.1300
4	15.5	3	6.9200	6.9200
4	15.5	4	7.3300	7.3300
5	30.8	1	5.7800	5.7800
5	30.8	2	4.6000	4.6000
5	30.8	3	6.4500	6.4500
5	30.8	4	5.7700	5.7700
6	64.9	1	6.2000	6.2000
6	64.9	2	5.8800	5.8800
6	64.9	3	5.3300	5.3300
6	64.9	4	5.4000	5.4000
7	135.7	1	3.8500	3.8500
7	135.7	2	4.5500	4.5500
7	135.7	3	3.8000	3.8000
7	135.7	4	3.9800	3.9800

Data listing File: BENDAPLN Date: 05-26-1992

FILTER: None

Obs.	CONC	REP	LENGTH
1	1	1	3.100000
2	1	1	3.940000
3	1	1	4.000000
4	1	1	4.040000
5	1	1	3.900000
6	1	2	3.680000
7	1	2	4.080000
8	1	2	4.140000
9	1	2	4.040000
10	1	2	3.860000
11	1	3	4.000000
12	1	3	3.960000
13	1	3	4.040000
14	1	3	4.180000
15	1	3	4.180000
16	1	4	3.940000
17	1	4	4.080000
18	1	4	3.900000
19	1	4	3.960000
20	1	4	4.120000
21	2	1	4.040000
22	2	1	3.820000
23	2	1	3.780000
24	2	2	4.320000
25	2	2	4.040000
26	2	2	4.000000
27	2	2	3.960000
28	2	2	3.760000
29	2	3	3.900000
30	2	3	3.960000
31	2	3	3.760000
32	2	3	3.760000
33	2	4	3.760000
34	2	4	3.720000
35	2	4	3.900000
36	3	1	4.360000
37	3	1	4.040000
38	3	1	4.000000
39	3	1	4.080000
40	3	2	4.140000
41	3	2	3.860000
42	3	2	4.040000
43	3	2	4.140000
44	3	2	4.320000
45	3	3	4.080000
46	3	3	3.680000
47	3	3	3.780000
48	3	3	4.140000
49	3	3	3.820000
50	3	4	4.080000
51	3	4	4.000000
52	3	4	3.960000
53	3	4	4.140000
54	3	4	4.140000
55	4	1	3.900000

Data listing File: BENDAPLN Date: 05-26-1992

FILTER: None

Obs.	CONC	REP	LENGTH
56	4	1	3.960000
57	4	1	4.040000
58	4	1	4.220000
59	4	1	4.260000
60	4	2	4.140000
61	4	2	3.760000
62	4	2	3.900000
63	4	2	4.140000
64	4	3	3.900000
65	4	3	4.120000
66	4	3	3.600000
67	4	3	3.780000
68	4	3	4.300000
69	4	4	4.040000
70	4	4	3.860000
71	4	4	4.080000
72	4	4	4.260000
73	5	1	4.120000
74	5	1	3.640000
75	5	1	3.860000
76	5	1	3.820000
77	5	1	3.600000
78	5	2	3.820000
79	5	2	3.820000
80	5	2	3.900000
81	5	2	3.760000
82	5	2	4.120000
83	5	3	4.080000
84	5	3	3.960000
85	5	3	3.900000
86	5	3	4.140000
87	5	3	3.940000
88	5	4	3.820000
89	5	4	3.680000
90	5	4	3.940000
91	5	4	3.960000
92	5	4	3.960000
93	6	1	3.760000
94	6	1	4.080000
95	6	1	4.000000
96	6	1	3.680000
97	6	1	4.080000
98	6	2	3.900000
99	6	2	3.900000
100	6	2	4.080000
101	6	2	3.900000
102	6	3	3.600000
103	6	3	3.720000
104	6	3	3.680000
105	6	3	3.900000
106	6	3	4.000000
107	6	4	3.780000
108	6	4	3.820000
109	6	4	3.820000
110	6	4	3.780000

Data listing File: BENDAPLN Date: 05-26-1992

FILTER: None

Obs.	CONC	REP	LENGTH
111	6	4	3.940000
112	7	1	3.600000
113	7	1	3.900000
114	7	1	3.640000
115	7	1	3.860000
116	7	1	3.720000
117	7	2	3.860000
118	7	2	3.860000
119	7	2	3.820000
120	7	2	3.820000
121	7	2	3.500000
122	7	3	3.600000
123	7	3	3.860000
124	7	3	3.820000
125	7	3	4.080000
126	7	3	3.680000
127	7	4	3.680000
128	7	4	3.780000
129	7	4	3.680000
130	7	4	3.820000
131	7	4	3.720000