

108501
SHAUGHNESSEY NO.

6-7-82
35
REVIEW NO.

EEB BRANCH REVIEW

DATE: IN 4/28/82 OUT 6/7/82

FILE OR REG. NO. 241-243

PETITION OR EXP. _____

DATE OF SUBMISSION April 13, 1982

DATE RECEIVED BY HED April 26, 1982

RD REQUESTED COMPLETION DATE August 16, 1982

EEB ESTIMATED COMPLETION DATE _____

RD ACTION CODE/TYPE OF REVIEW 400/

TYPE PRODUCT(S): I, D, H, F, N, R, S Herbicide

DATA ACCESSION NO(S). _____

PRODUCT MANAGER NO. R. Taylor (25)

PRODUCT NAME(S) PROWL

COMPANY NAME American Cyanamid Company

SUBMISSION PURPOSE Submission of Chronic Daphnia Study in Support of
Registration.

SHAUGHNESSEY NO.	CHEMICAL & FORMULATION	% A.I.
<u>108501</u>	<u>(N-(1-ethylpropyl)-3,4-dimethyl-2,</u>	_____
_____	<u>6-dinitrobenzenamine)</u>	_____
_____	_____	_____
_____	_____	_____

PROWL

100. Pesticide Label Information

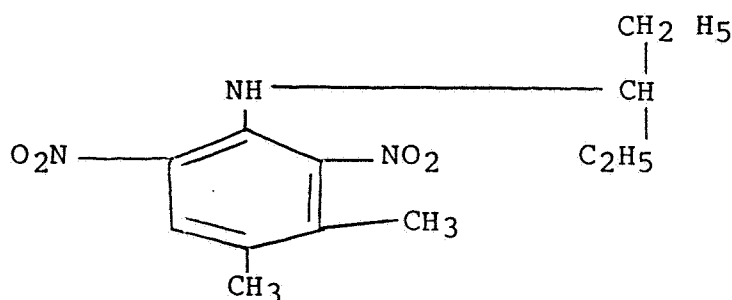
This submission contains only data requests made in connection with conditional registration. Therefore, no labeling was submitted.

101. Physical and Chemical Properties

101.1 Chemical Name

(N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine)

101.2 Structural Formula



101.3 Common Names

Penoxyn, Penoxalin, Pendimethalin, AC92,553
CL92,553

101.4 Trade Name

Prowl

101.5 Molecular Weight

281.3

101.6

Physical State

Color and State Orange-yellow crystals

Odor Faint, nutty odor

Boiling point 330°C

Melting point 56-57°C

Specific Gravity 1.9 @ 25°C

Vapor Pressure 3.0×10^{-5} mm Hg @ 25°C

Stability Stable to Alkaline and
Acidic Conditions

Corrosiveness Non-corrosive

101.7

Solubility

<u>Solvent</u>	<u>Temp. °C</u>	<u>Solubility (g/l)</u>
Water	23	0.5
Acetone	26	699
Xylene	26	628
Isopropanol	26	77
Corn oil	26	148

107.

Conclusions

107.4

Data Adequacy Conclusions

The attached study entitled, Chronic (21-Day) toxicity of AC92, 553 to Daphnia magna Straus, does not meet guideline requirements in that the grade of the chemical tested was not identified or was the percent of active ingredient given.

See 9-3-82 memo.
on change in status.
12-2-82 JFW

Dennis J. McLane, Wildlife Biologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769)

Dennis J. McLane 6-5-82

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TS-769:EEB:DJMcLane:gs:X79307:CM#2:RM1128:6/7/82

DATA EVALUATION RECORD

1. CHEMICAL: (N-(1-Ethylopropyl)-3,4-dimethyl-2,6-dinitrobenzenamine)
[Prowl/Pendimethalin]
2. FORMULATION: Not reported
3. CITATION: Gramey, R.L. 1981. The chronic (21-day) toxicity of AC 92,553 to Daphnia magna Straus. Biospherics Inc. Project No. 5179, submitted by American Cyanamid Co., Agricultural Division, Princeton, N.J. for EPA Reg. No. 241-243 on 4/20/82, CDL Acc. No. 247299.
4. REVIEWED BY: Dennis J. McLane, Wildlife Biologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769)
5. DATE REVIEWED: May 20, 1982
6. TEST TYPE: Daphnia magna life-cycle toxicity
7. REPORTED RESULTS: The LC₅₀'s calculated for 4 and 8 days respectively, were 32.5 (23.6-44.7) and 19.1 (confidence limits unobtainable) ug/l. The 15 and 21 day LC₅₀ values were 18.2 ug/l and 19.0 ug/l, respectively. Both values were corrected to compensate for control mortality. After 15 day and 21 days the 22.1 and 17.2 ug/l levels, respectively, reduced cumulative production by 50% (RI₅₀). The calculated 10, 15 and 21 day no observed effect concentration (NOEC), based on total production was 14.5 ug/l.
8. REVIEWER'S CONCLUSIONS: This study is scientifically sound. However, it does not meet the guideline requirements. The report fails to indicate the percent active ingredient or the grade of the chemical.

SEE 7-5-82 memo by D. McLane
for change in status.
J. [unclear]
12-3-82

B. Statistical Analysis

The LC₅₀'s were determined by the Litchfield and Wilcoxon Method (1949). Abbott's formula was used to adjust the the LC₅₀ values to determine the affect of control mortality.

The percent reproductive impairment, R.I.₅₀ was determined by the Litchfield and Wilcoxon Method (1949).

The effect of different treatment levels on Daphnia brood sizes and cumulative young produciton were determined using Analysis of Variance (ANOVA) and Duncans Multiple Range Test.

The No Observed Effect Concentration (NOEC) was calculated as the highest concentration tested in which the number of young produced did not differ significantly from that in the controls, as determined by Duncans Multiple Range Test.

C. Discussion/Results

1. LC₅₀'s, ug/l

		Day 4	Day 8	Day 15		Day 21	
				U	C	U	C
LC ₅₀		32.5	19.1	17.2	18.2	17.2	19.0
95%	Lower	23.6	N.O.	15.2	N.O.	15.2	N.O.
Confi- dence Limits	Upper	44.7	N.O.	19.4	N.O.	19.4.	N.O.

2. RI₅₀: 15 Day RI₅₀ - 22.1 ug/l
21 Day RI₅₀ - 17.2 ug/l

Effect on Overall Survival:

By day 6, 100% mortality had occurred in the two highest concentrations (35.8 and 74.2 ug/l). At test termination, there was no appreciable mortality at the three lowest exposure levels (4.3, 8.2 and 14.5 ug/l).

3. Effects on Overall Productivity:

Duncans Multiple Range Test indicates that cumulative production means for control, solvent control and 3 treatments (4.3, 8.2 and 14.5 ug/l) were not significantly different.

4. Effect on Mean Brood Sizes:

Duncans Multiple Range Test indicates that AC 92,553 had no significant effect on mean brood size.

5. Calculated NOEC: 14.5 ug/l.

N.O. - Not obtainable.

U - Uncorrected for control mortality.

C - Corrected for control mortality.

Reviewer's Evaluation

A. Test Procedures

The author made the following statements concerning deviations from the protocol:

1. The temperature range was 21°C - 22°C as opposed to protocols stated temperature of 20 ± 1°C.
2. The water acidity was not measured.
3. Analytical determinations of the concentrations were made on days 0, 1, 3, 7, 10, 14, 17 and 21, with each stock solution being analytically verified. The protocol stated that analytical samples were to be frozen and analyzed at the end of the test, with stock solutions being analyzed weekly.
4. Flow rate during the test resulted in, approximately 2.8 turnovers per day as opposed to protocol stated for 4-6 to turnovers.
5. Diluter operation (i.e., test volume delivery, number of cycles, etc.) was checked daily, however, not recorded.

6. The No Observed Effect Concentration (NOEC) was calculated instead of the Maximum Acceptable Toxicant Concentration (MATC); however, for this study they are numerically the same.

In addition to these amendments to the protocol (ASTM Draft No. 3 March 1981) there was no mention of a 21 holding period prior to the study, nor did the report indicate, prior to starting a test, that Daphnia which are at least 10-12 days old (those which have had at least one brood) should be separated from the culture, put in a separate culture container and maintained as described above. Also, the report indicates 5 of the instars were selected and distributed at random to each test vessel, a total of 20 daphnids for each toxicant concentration and control. The protocol indicates that each concentration is replicated 4 times using 10 Daphnia per replicate yielding a total of 40 Daphnia per treatment.

The test material formulation was not given, that is, the chemical grade and percent active ingredient.

B. Statistical Analysis

Four portions of the study required statistical interpretation. First, the LC₅₀ for days 4, 8, 15, and 21 were calculated. These were verified by EEB's Stephan's computer program (see the attached results). The resulting values did not significantly vary from Biospherics.

The next two items require ANOVA interpretations: cumulative young product and mean brood sizes. EEB's SAS ANOVA and Duncans Multiple Range Test are in agreement with Biospherics, that the treatment level of 14.5 ug/l and below are not significantly different from the control. However, it should be mentioned that for the 17-21 day interval for the mean brood size for the 14.5 ug/l level was significantly different from the solvent control, 4.3, and 8.2 ug/l but not the control.

Lastly, the RI₅₀ for 21 days was verified by the Stephan's program (see attached printout). This value 19.9 ug/l (C.L. of 14.5 to 35.8 ug/l for the binomial method) is not significantly different than the reported value of 22.1 ug/l.

Discussion/Results

The study presents a relative comparison of the chronic effects to Daphnia. Though the holding period was not reported, test dates indicated a 21 day period. However, the test material formulation is required.

Conclusion:

1. Category - Supplemental
2. Rationale - The study is scientifically sound. It does not meet the guideline requirements. The study failed to report the formulation (percent of active ingredient or grade of the chemical).
3. Repairability - Yes, provide the formulation for the test material.

Daphnia LC₅₀ uncorrected for control mortality (ug/l)Day 4

Binomial LC₅₀ 31.99

Moving Average LC₅₀ 34.37 C.L. 27.9 - 44.3

Probit LC₅₀ 31.29 C.L. 0 - infinity

Day 8

Binomial LC₅₀ 21.5

Moving Average LC₅₀ 17.7

Probit LC₅₀ 17.6 C.L. 0 - infinity

Day 15

Binomial LC₅₀ 19.91 C.L. 14.5 - 35.8

Moving Average LC₅₀ 16.2 C.L. 12.8 - 20.62

Probit LC₅₀ 16.3 C.L. 0 - infinity

Day 2

Binomial LC₅₀ 19.2 C.L. 14.5 - 35.8

Moving Average LC₅₀ 14.86 C.L. 11.3-19.34

Probit LC₅₀ 14.77 C.L. 0 - infinity

MCLANE PROWL

RI 50

21 days

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
35.8	901	901	100	0
14.5	901	120	13.31853	0
8.2	901	0	0	0
4.3	901	33	3.662597	0

THE BINOMIAL TEST SHOWS THAT 14.5 AND 35.8 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 19.86833

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
11	35.69536	2348.07	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 5.370687
95 PERCENT CONFIDENCE LIMITS = -26.7168 AND 37.45818

LC50 = 19.01054
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 11.02873
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

McLURE PROWL DAPHNIA LC50

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CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
           EXPOSED     DEAD        DEAD        PROB.(PERCENT)
74.2       18          18          100          0.0003814697
35.8       18          18          100          0.0003814697
14.5       18          2           11.1111     0.06561279
8.2        18          1           5.5556      0.007247925
4.3        18          1           5.5556      0.007247925
  
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THE BINOMIAL TEST SHOWS THAT 14.5 AND 35.8 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 20.49941

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	0.0528356	18.4985	14.70402	23.662

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	2.742576	8.212363	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.425728
95 PERCENT CONFIDENCE LIMITS = -2.903598 AND 11.75505

LC50 = 18.29986
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 9.451252
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Day 21

PROWL DAPHNIA LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
74.2	17	17.	100	0.0007629395
35.8	17	17	100	0.0007629395
14.5	17	1	5.8824	0.01373291
8.2	17	0	0	0.0007629395
4.3	17	1	5.8824	0.01373291

THE BINOMIAL TEST SHOWS THAT 14.5 AND 35.8 CAN BE USED AS STATISTICALY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 21.328

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	0.05504922	21.07382	16.68025 27.50513

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	10.48471	28.37556	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CAMCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE°USED.

SLOPE = 4.810362
95 PERCENT CONFIDENCE LIMITS = -10.76564 AND 20.38636

LC50 = 19.7356
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 10.74575
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Methods and Materials

A. Test Procedures

Parameter	Measure, Setting or Condition
1. Test material	AC 92,553 (Prowl)
2. Test type	<u>Daphnia magna</u> 21-Day Chronic (flow-through)
3. Test date	7/16/81 - 9/8/81
4. Physical test apparatus, toxicant injection system	Ace Glass solenoid diluter with teflon fittings and tubing. Direct flow toxicant delivery system.
5. Nominal toxicant stock solution concentration.	75.0 µg/l
6. Cumulative Mean Measured test water concentration	4.3, 8.2, 14.5, 35.8 and 74.2 µg/l plus control and solvent control (0.5 mg/l acetone)
7. Test vessels	Twenty-eight rectangular battery jars (10 x 10 x 20 cm; 1.3 liter capacity).
8. Dilution water and volume	Biospherics Incorporated site well water; 20 minute cycle time; 50 ml per replicate per cycle.
9. Mean water quality characteristics	Total hardness 115 mg/l as CaCO ₃ , total alkalinity 147 mg/l as CaCO ₃ , conductivity 352 µmhos/cm, pH range 7.3 - 7.6.
10 Photoperiod	Sixteen hours light, eight hours darkness. Light provided by General Electric Gro and Sho fluorescent lights (Color rendering index >90). Surface light intensity 575 - 625 lux.
11. Bioassay organism	<u>Daphnia magna</u> Straus obtained from laboratory stock culture. Twenty organisms per concentration (5 per replicate).
12. Feeding rate, food	40 mg/test vessel/day (Trout chow and yeast suspension).
13. Mortality and productivity counts	Counts made on days 1, 2, 3, 4, 6, 8, 10, 13, 15, 17 and 21. Dead individuals and instars removed on these days.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	4	128.80300000	32.20075000	3.73
ERROR	15	129.44500000	8.62966667	PR > F
CORRECTED TOTAL	19	258.24800000		<u>0.0267</u>

R-SQUARE	C.V.	STD DEV	RESPONSE MEAN
0.498757	20.3156	2.93762943	14.46000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
VARIABLE	4	128.80300000	3.73	0.0267

SOURCE	DF	TYPE IV SS	F VALUE	PR > F
VARIABLE	4	128.80300000	3.73	<u>0.0267</u>

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RESPONSE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=15 MS=8.62967

GROUPING	MEAN	N	VARIABLE
A	16.700000	4	U
A	16.375000	4	T
A	16.150000	4	S
A	12.875000	4	R
A	10.200000	4	V

14.5