<u>254841</u>
RECORD NO.

<u>031301</u>
SHAUGHNESSEY NO.

1/17/90

REVIEW NO. _____

<u>EEB REVIEW</u>

DATE: IN __<u>11-20-89</u>__   OUT _____

FILE OR REG. NO. _____<u>45639-128</u>_____

PETITION OR EXP. NO. _____

DATE OF SUBMISSION _____

DATE RECEIVED BY EFED _____<u>11-13-89</u>_____

RD REQUESTED COMPLETION DATE ___<u>01-13-90</u>_____

EEB ESTIMATED COMPLETION DATE ___<u>01-13-90</u>_____

RD ACTION CODE/TYPE OF REVIEW ___<u>660</u>_____

_____

TYPE PRODUCT(S) _____<u>Fungicide</u>_____

DATA ACCESSION NOS. _____<u>405831-02</u>_____

PRODUCT MANAGER NO. _____<u>E. Feris (21)</u>_____

PRODUCT NAME(S) _____<u>DCNA (Dichloran) Technical</u>_____

_____

COMPANY NAME _____<u>NOR-AM Chemical Company</u>_____

SUBMISSION PURPOSE _____<u>Submitted in response to RS</u>_____

_____

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% AI
<u>031301</u>	<u>Dichoran</u>	<u>97.0</u>

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

Subject: Review of Freshwater Invertebrate Acute Toxicity Test
(Daphnia magna) for DCNA (Dichloran Technical)

From: James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

To: Eric Feris (PM-21)
Reregistration Branch
Special Review/Reregistration Division (H7508C)

Attached is the Ecological Effects Branch review of a freshwater
invertebrate acute toxicity study using Daphnia for the
reregistration of DCNA.

The study is scientifically sound with a few minor deviations
from standard methodologies. This study fulfills the basic
requirements of current guidelines and would be acceptable for
use in a risk assessment. The 48-hour EC_{50} with 95% confidence
limits for the toxicity of Dichloran Technical to Daphnia magna
was calculated to be 2.07 mg/l. This value places Dichloran
Technical in a category of moderately toxic to freshwater
invertebrates.

DATA EVALUATION RECORD

1. **CHEMICAL:**   DCNA

2. **TEST MATERIAL:** Dichloran Technical (97% a.i.),
   a yellow powder

3. **STUDY TYPE:**   Freshwater Invertebrate Acute Toxicity Test
   Species Tested: <u>Daphnia magna</u>

4. **STUDY IDENTIFICATION:**

   Author: Hill, R.W., Moffat, A.M., and M.H.I. Comber
   Laboratory: The Brixham Laboratory
   Chester Park Research Station
   Saffron Walden, Essex England
   Study No.: ENVIR/88/19
   Study Date: 03/11/88
   Submitted By: NOR-AM Chemical Company
   Accession No: 40583102

5. **REVIEWED BY:**

   Art Roybal   Signature: _Art Roybal_
   Wildlife Biologist
   Ecological Effects Branch, EFED   Date: 1-12-90

6. **APPROVED BY:**

   Norman Cook   Signature: _Norman J. Cook_
   Supervisory Biologist
   Ecological Effects Branch, EFED   Date: 1.17.90

7. **CONCLUSIONS:** The study is scientifically sound with a few minor deviations from standard methodologies. This study fulfills the basic requirements of current guidelines and would be acceptable for use in a risk assessment. The 48-hour EC_{50} with 95% confidence limits for the toxicity of Dichloran Technical to <u>Daphnia magna</u> was calculated to be 2.07 mg/l. This value places Dichloran Technical in a category of moderately toxic to freshwater invertebrates.

8. **RECOMMENDATIONS:** N/A

9.   BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A.   Test Animals: The test organism was the freshwater crustacean, Daphnia magna, obtained from continuous laboratory cultures. The stock cultures of Daphnia were maintained in a reccnstituted water medium, identical to the test dilution water, at a temperature of $20 \pm 2^\circ C$, and a photoperiod of 16 hours light: 8 hours dark. The cultures were fed a defined diet of algae and yeast. Daphnia less than 24 hours old were used for testing. (From brood stock 17 days old. No symptoms of disease had been observed in this culture).

B.   Preparation of Test Solution: A 10 ml stock solution of the test substance was prepared in acetone (11,200 mg/l). The test concentrations were prepared by the addition of aliquots of this stock solution to the dilution medium using microlitre syringes. The test solutions were stirred for one hour prior to use. The following nominal exposure concentrations were employed: control, solvent control, 0.32, 0.56, 1.0, 1.8, 3.2, and 5.6 mg/l dichloran technical. Apart from the control, each exposure concentration contained 500 ul/l of acetone.

C.   Test Water: The water was aerated for greater than 2 hours before use. The pH of the dilution water was 8.42.

D.   Test Procedure: Borosilicate glass beakers (250 ml) were used as test vessels, with four replicates per exposure concentration, each containing 200 ml of test solution. When the test solutions were at test temperature, five Daphnia were randomly added to each test vessel in <2.0 ml of dilution water, giving a total of 20 Daphnia per concentration. A random number table was used for this purpose. The temperature was maintained at $20 \pm 1^\circ C$ with a photoperiod of 16 hours light: 8 hours dark. The test vessels were not aerated during the course of the test.

E.   Observation Of Effects: Assessments of the response of the Daphnia were made at 24 and 48 hours after commencement of the test. Each Daphnia was viewed by eye, and was defined as affected if showing no whole body movement relative to the water within a period of 15 seconds, even if movement of individual appendages was visible. Daphnia so affected were termed immobile.

F.   Physical and Chemical Analysis: The dissolved oxygen concentration of the control (dilution water used to prepare the test solution) was measured prior to the start of the test. The initial pH of each test solution was determined

using the excess remaining after filling the test vessels. At the end of the test the pH and dissolved oxygen concentration of two replicates of each control and treatment were measured. The temperature of the water in an additional test vessel containing no Daphnia was measured by thermometer at 0, 24 and 48 hours, and at hourly intervals by an automatic recording system (platinum resistance sensor). The concentration of the test solution was measured at the start (excess solution) and end of the test by the method described in Appendix 2. At the end of the test one replicate of each test concentration was sampled.

F. **Statistics**: Statistical analysis of the concentration vs. effect data was obtained by using Stephan et al. (1978), a computerized LC_{50} program. This program calculated the LC_{50} and its 95% confidence limits by using the binomial, moving average and probit tests. All calculations were based on the nominal concentrations of Dichloran Technical.

12. **REPORTED TEST RESULTS:**

<u>Biological Data</u>: The number of Daphnia immobilized at each concentration, after 24 and 48 hours, are given in Table 1. The authors state, "No symptoms of toxicity were observed in this study." The numbers immobilized in the replicates were summed for each treatment and control during each time period. Where the data permitted, the 24 and 48 hour median effective concentrations and their 95% confidence limits were calculated by probit analysis using a computer program. A graph of the concentration:percentage response after 48 hours, showing the regression line fitted by the analysis, is given in Figure 1. The median effective concentration (EC_{50}) was defined as the concentration, calculated from the data obtained, resulting in 50% immobilization of the Daphnia in the time period specified. The results obtained were:

	EC_{50}	Confidence Limits
24 hours (slope of probit = 4.2)	4.43	3.62 - 6.03 mg/l
48 hours (slope of probit = 8.6)	2.07	1.80 - 2.39 mg/l

<u>Chemical and Physical Analysis</u>: It was noted in this study that all test exposure concentrations appeared to be clear and no precipitation of the test material was observed. The levels of Dichloran in the exposure vessels were determined on all exposure concentrations and the Daphnia media control and solvent control by high performance liquid chromatography at 0 and 48 hours. Dissolved oxygen levels ranged from 9.0 to 9.3 mg/l and the pH values ranged from 8.28 to 8.42. The temperature recorded by thermometer at 0,

24 and 48 hours were 20.3, 20.5, and 20.3°C respectively (see Table 3). The range of temperatures recorded automatically at hourly intervals was 20.0 to 20.5°C. The concentrations of the test substance determined in the test solutions at the start and finish of the test are given in Table 2. The measured concentrations at the start of the test ranged from 91 to 110% of the nominal values. The measured concentrations at the end of the test ranged from 96 to 110% of the nominal value, therefore, the nominal values were used in this report.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES**: "The 24 hour and 48 hour EC_{50} values were determined to <u>Daphnia magna</u> using dicloran technical. The 48-hour EC_{50} value was 2.07 mg/l dicloran technical based on nominal concentration. According to the Standard Evaluation Procedure this formulation would be classified as moderately toxic."(This is a direct quote including spelling, etc.)

The authors state the study was conducted following the intent of the Good Laboratory Practice Regulations.

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

A. **Test Procedure**: The study is in general accordance with EPA Standard Evaluation Procedures and ASTM procedures.

B. **Statistical Analysis**: The EPA Toxanal computer program was used to verify EC_{50} values at the 24 and 48 hour intervals. EC_{50} values obtained with the EPA Toxanal program coincide with the values calculated in this study. See attached documentation.

C. **Discussion/Results**: A 48-hour EC_{50} value of 2.07 mg/l would place Dichloran Technical in the classification of moderately toxic to <u>Daphnia magna</u>.

D. **Adequacy of the Study**:

(1) **Classification**: Core

(2) **Rationale**: Minor inconsistencies with standard methodologies, however, the deviations do not detract from the study's soundness or intent

(3) **Repairability**: N/A

15. **COMPLETION OF ONE-LINER FOR STUDY**: Yes, 01/12/90

Roybal dichloran technical Daphnia 01-10-90   *24- Hour*
****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
5.6	20	13	65	13.1588
3.2	20	6	30	5.765915
1.8	20	1	5	2.002716E-03
1	20	0	0	9.536742E-05
.56	20	0	0	9.536742E-05
.32	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT   0   AND +INFINITY 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 4.412896

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.8069815	4.412896	2.870203	9.605119

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.2013353	1	.9975969

SLOPE   =   4.183476
95 PERCENT CONFIDENCE LIMITS = 2.306334   AND   6.060619

LC50 =   4.428219
95 PERCENT CONFIDENCE LIMITS = 3.615435 AND 6.025872

LC10 =   2.201171
95 PERCENT CONFIDENCE LIMITS = 1.361506 AND 2.779418
****

```
******************************************************************
CONC.     NUMBER        NUMBER       PERCENT     BINOMIAL
          EXPOSED       DEAD         DEAD        PROB.(PERCENT)
 5.6        20            20          100        9.536742E-05
 3.2        20            19           95        2.002716E-03
 1.8        20             6           30        5.765915
 1          20             0            0        9.536742E-05
 .56        20             0            0        9.536742E-05
 .32        20             0            0        9.536742E-05
```

THE BINOMIAL TEST SHOWS THAT 1 AND 3.2 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 2.107342

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
```
SPAN        G           LC50          95 PERCENT CONFIDENCE LIMITS
 4      5.135013E-02              2.076829        1.747534
2.508648
```


RESULTS CALCULATED USING THE PROBIT METHOD
```
ITERATIONS      G        H       GOODNESS OF FIT PROBABILITY
    7       .1975888     1            .9995357
```

SLOPE   =   8.92993
95 PERCENT CONFIDENCE LIMITS = 4.96049   AND   12.89937

LC50 =   2.074524
95 PERCENT CONFIDENCE LIMITS = 1.804893 AND 2.390401

LC10 =   1.495203
95 PERCENT CONFIDENCE LIMITS = 1.100391 AND 1.733395
****

Study/Species/Lab/ Access. #	Chemical % a.i	Results	Reviewer/ Date	Validation Status

14-Day Single Dose Oral LD$_{50}$,

Species;

Lab.;

Acc. #;

LD50 = ___ mg/kg (_____ 95% C.L ___)   Contr. Mort.(%)=

Slope=   # Animals/Level=   Age(Days)=   Sex =

14-Day Dose Level mg/kg/(% Mortality)
( ), ( ), ( ), ( ), ( )

Comments:

14-Day Single Dose Oral LD$_{50}$,

Species;

Lab.;

Acc. #;

LD50 = ___ mg/kg (_____ 95% C.L ___)   Contr. Mort.(%)=

Slope=   # Animals/Level=   Age(Days)=   Sex =

14-Day Dose Level mg/kg/(% Mortality)
( ), ( ), ( ), ( ), ( )

Comments:

8-Day Dietary LC$_{50}$,

Species

Lab.;

Acc. #

LC50 = ___ ppm (_____ 95% C.L. ___)   Contr. Mort.(%)=

Slope=   # Animals/Level=   Age(Days)=   Sex =

8-Day Dose Level ppm/(%Mortality)
( ), ( ), ( ), ( ), ( )

Comments:

8-Day Dietary LC$_{50}$,

Species;

Lab;

Acc. #

LC50 = ___ ppm (_____ 95% C.L ___)   Contr. Mort.(%)=

Slope=   # Animals/Level=   Age(Days)=   Sex =

8-Day Dose Level ppm/(%Mortality)
( ), ( ), ( ), ( ), ( )

Comments:

96-hour LC$_{50}$,

Species;

Lab;

Acc. #;

LC50 = ___ PP__ (_____ 95% C.L. ___)   Contr. Mort.(%)=   Sol. Contr. Mort.(%)=

Slope=   # Animals/Level=   Temperature =

96-Hour Dose Level pp /(%Mortality)
( ), ( ), ( ), ( ), ( )

Comments:

96-hour LC$_{50}$,

Species;

Lab.;

Acc. #;

LC50 = ___ PP__ (_____ 95% C.L. ___)   Con. Mor(%)= _   Sol. Con. Mor.(%)=

Slope=   # Animals/Level=   Temp.=

96-Hour Dose Level pp /(%Mortality)
( ), ( ), ( ), ( ), ( )

Comments:

48-hour Invertebrate,

Species: Daphnia

Lab.: Brixham Lab ——— 77% a.i.

Acc. #: H058310 Z

LC50 = 2.07 ppm (1.80 - 2.39 95% C.I.)   Con. Mort.(%)= 0   Sol. Con. Mort.(%)= 0

Slope= 8.93   # Animals/Level= 20   Temp.= 20.3   label CORE 1:?:?0

96-Hour Dose Level ppm /(%Mortality)
.32 (0%), .56 (5%), 1.0 (0%), 1.8 (?%), 3.2 (95%), 5.6 (100%)

Comments:

Shaughnessey No. _____

Chemical Name _____ Chemical Class_____ Page __2__ of _____

| Study/Species/Lab/ Accession # | Chemical % Active | | | Reviewer/ Date | Validation Status |

Avian Reproduction,

Species:

Results

Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%ChE Inh.
Control	_____	_____	_____	_____
Treatment I	_____	_____	_____	_____
Treatment II	_____	_____	_____	_____
Treatment III	_____	_____	_____	_____

Lab:

Acc. #:

Study Duration:

Comments:

Field Study(Simulated/Actual)

Species:

Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)
Control	_____	_____	_____	_____
Treatment I	_____	_____	_____	_____
Treatment II	_____	_____	_____	_____
Treatment III	_____	_____	_____	_____

Lab:

Acc. #:

Crop/Site:

Study Duration:

Comments:

Chronic fish,

Species

Lab:

Acc.#:

Concentrations Tested (pp__)= _____

MATC = >____ <_____ pp___. Effected Parameter = _____

Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____

Comments:

Chronic invertebrate,

Species

Lab:

Acc.#:

Concentrations Tested (pp__)= _____

MATC =>____< _____pp___. Effected Parameter(s)_____

Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____

Comments:

⊕EPA

United States Environmental Protection Agency
Office of Pesticide Programs
Washington, DC 20460

Data Review Record

Confidential Business Information - Does not contain
National Security Information (E.O. 12065)

0313O

Pack Number	Date Received
49820 EFED	11-13-89

1. Product Name

Chemical Name DCNA

2. Identifying Number	3. Record Number	4. Action Code	5. MRID/ Accession Number	6. Study Guideline or Narrative
45639-128	254841	660	40583102	72-2 A

7. Reference No.	8. Date Rec'd (EPA)	9. Prod/Review Mgr/DCI	10. PM/RM Team No.	11. Date to HED/ EFED/RD/BEAD	12. Proj Return Date	13. Date Returned to RD/SRRD
	4-12-88	E. FERIS	21	11-13-89	1-13-90	

Instructions

Please review data

031301-5/0113

This Section Applies to Review of Studies Only

14. Check Applicable Box

- [ ] Adverse 6(a)(2) Data (405)
- [ ] Special Review Data (870)
- [✓] Generic Data (Reregistration)(660)
- [ ] Product Specific Data (Reregistration)(655)

15. No. of Individual Studies Submitted
1

16. Have any of the above studies *(in whole or in part)* **been previously submitted for review?**

- [ ] Yes *(Please identify the study(ies))*
- [ ] No

17. Related Actions

18.	To	Type of Review	19. Reviews Also Sent to		20. Data Review Criteria
HED		Science Analysis & Coordination	[ ] SAC	[ ] PC	**A. Policy Note No. 31**
		Toxicology/HFA	[ ] TOX/HFA	[ ] PL	
		Toxicology/IR	[ ] TOX/IR		[ ] 1 = data which meet 6(a)(2) or meet 3(c)(2)(B) flagging criteria
		Dietary Exposure	[ ] DEB	[ ] EA	
		Nondietary Exposure	[ ] NDE	[ ] AC	
EFED	[✓]	Ecological Effects		[ ] BA	[X] 2 = data of particular concern from registration standard
		Environmental Fate & Groundwater	[ ] EEB		
SRRD		Special Review	[ ] EFGWB		
		Reregistration			[ ] 3 = data necessary to determine tiered testing requirements
		Generic Chemical Support	[ ] SR		
RD		Insecticide-Rodenticide	[ ] RER		
		Fungicide-Herbicide	[ ] GSC		**B. Section 18**
		Antimicrobial			[ ] 1 = data in support of section 3 in lieu of section 18
		Product Chemistry	[ ] IR		
		Precautionary Labeling	[ ] FH		
BEAD		Economic Analysis	[ ] AM		**C. Inert Ingredients**
		Analytical Chemistry			[ ] 1 = data in support of continued use of List 1 inert
		Biological Analysis			

- [ ] Confidential Statement of Formula (EPA Form 8570-4) Attached *(Trade Secrets)*
- [ ] Label Attached

EPA Form 8570-17 (Rev. 11-88)
Previous editions are obsolete.

White - Data Coordinator
Yellow - Data Review Section

Pink - PM/RM/DCI
Green - Return with completed review

Use this form for individual studies & to submit pesticide applications.

		Pack Number	Date Received
United States Environmental Protection Agency Office of Pesticide Programs Washington, DC 20460 **Data Review Record** *Confidential Business Information - Does not contain* *National Security Information (E.O. 12065)*		49820 *EFED*	11-13-89

EPA

1. Product Name	Chemical Name
	DCNA

2. Identifying Number	3. Record Number	4. Action Code	5. MRID/ Accession Number	6. Study Guideline or Narrative
45639-128	254841	660	40583102	72-2A

7. Reference No.	8. Date Rec'd (EPA)	9. Prod/Review Mgr/DCI	10. PM/RM Team No.	11. Date to HED/ EFED/RD/BEAD	12. Proj Return Date	13. Date Returned to RD/SRRD
	4-12-88	E. FERIS	21	11-13-89	1-13-90	

Instructions

Please review data

031301-5/0113

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☑ Generic Data (Reregistration)(660)
☐ Special Review Data (870)
☐ Product Specific Data (Reregistration)(655)

15. No. of Individual Studies Submitted 1

16. Have any of the above studies *(in whole or in part)* been previously submitted for review?

☐ Yes *(Please identify the study(ies))*   ☐ No

17. Related Actions

18.	To	Type of Review	19. Reviews Also Sent to		20. Data Review Criteria
HED		Science Analysis & Coordination	☐ SAC	☐ PC	A. Policy Note No. 31
		Toxicology/HFA	☐ TOX/HFA	☐ PL	
		Toxicology/IR	☐ TOX/IR		☐ 1 = data which meet 6(a)(2) or meet 3(c)(2)(B) flagging criteria
		Dietary Exposure	☐ DEB	☐ EA	
		Nondietary Exposure	☐ NDE	☐ AC	
EFED	✓	Ecological Effects		☐ BA	☒ 2 = data of particular concern from registration standard
		Environmental Fate & Groundwater	☐ EEB		
SRRD		Special Review	☐ EFGWB		☐ 3 = data necessary to determine tiered testing requirements
		Reregistration			
		Generic Chemical Support	☐ SR		
RD		Insecticide-Rodenticide	☐ RER		B. Section 18
		Fungicide-Herbicide	☐ GSC		
		Antimicrobial			☐ 1 = data in support of section 3 in lieu of section 18
		Product Chemistry	☐ IR		
		Precautionary Labeling	☐ FH		
BEAD		Economic Analysis	☐ AM		C. Inert Ingredients
		Analytical Chemistry			☐ 1 = data in support of continued use of List 1 inert
		Biological Analysis			

☐ Confidential Statement of Formula
(EPA Form 8570-4) Attached *(Trade Secrets)*   ☐ Label Attached

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