3-10-93

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

MAR 10 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Memorandum:

Subject: Aquatic Plant toxicity testing with Bromacil

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division(H7507C)

To: Linda Propst, Product Manager 73
Special Review and Reregistration Division(H7508W)

EEB has completed review of the Bromacil Tier II aquatic plant study using Selenastrum capricornutum(MRID 425164-01). The study, submitted by E. I. Du Pont does fulfill Tier II testing requirements. EEB found statistically significant reductions in algal cell counts at all concentrations tested. The EC_{50} results are useable in the risk assessment process. Therefore the study is considered acceptable. The EC_{50} of 6.8 ppb classifies this herbicide as very highly toxic to freshwater algae. The NOEC was not clearly established by the study, but is estimated to be < 1.1 ppb.

ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT

1. **Chemical:** Bromacil **Shaughnessy Code:** 012301

2. **Test Material:** Bromacil technical, 96.5% ai, Lot T07128852

3. **Study Type:** Tier II Aquatic Plant Toxicity to Selenastrum capricornutum.

4. **Study Identification:**
   Study Director: Williams, Toni L.
   Study Laboratory: Malcolm Pirnie, Inc.
   Study Dates: June 5-10, 1992
   Study Identification: Study No. B382-156-1
   Study Sponsor: E. I. Du Pont, Agricultural Product Div.
   EPA Identification: MRID 425164-01

5. **Reviewed by:** Brian Montague, Fisheries Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division(H7507C)

   Brian Montague 3/2/93

6. **Approved by:** Les Touart, PhD, Section Supervisor
   Ecological Effects Branch
   Environmental Fate and Effects Division

   L. Touart 3/2/93

7. **Conclusions:** The study was conducted with sound scientific methodology and results are useable for risk assessment purposes. The study does fulfill reregistration data requirements The EC_{50} of 6.8(5.9-7.8) ppb established by the study shows the chemical to be highly toxic to the freshwater green algae, Selenastrum capricornutum. The NOEC was not established in this study as all concentrations produced an effect on algal populations. The NOEC will occur at some point < 1.1 ppb, the lowest concentration tested.

8. **Recommendations:** Though the study should be repeated at a lower dose range to establish the dosage level at which there is no effect on survival of cell populations(NOEC), this is not required at this time.

9. **Submission Purpose:** Submitted to satisfy reregistration data requirements.

✓ 2

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, *Selenastrum capricornutum*, came from laboratory stock cultures originally obtained from the University of Texas Culture Collection, Austin, TX. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP) under 4306 lux illumination, and a temperature of 24 ±2°C. The cultures were continuously shaken at 100 rpm. Transfers were made regularly to provide logarithmically-growing cultures. The culture used as inoculum in this test had been transferred to fresh medium seven days before test initiation.

B. **Test System:** All glassware were cleaned and autoclaved before use. Test vessels used were 250-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ±0.1. The medium was filter sterilized (0.22 µm) prior to inoculation.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing (24 ±2°C, 100 rpm shaking) with continuous cool-white illumination (4306 ±646 lux).

A 0.7 mg/ml stock solution was prepared by dissolving 33.7 mg of the test material in deionized water to a volume of 50 ml. Secondary stocks (0.005 and 0.0005 mg/ml) were prepared by serial dilution of the primary stock with deionized water. The test solutions were created by addition of appropriate volumes of the 0.0005 mg/ml stock solution to 250 ml of nutrient medium.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, five nominal concentrations of 1.25, 2.5, 5, 10, and 20 µg/l were selected for the definitive test. A medium control was also prepared. The maximum labeled application rate for bromacil is 24 lb active ingredient/acre. This is equivalent to 17.64 mg/l if applied to a six-inch water column.

D. **Test Design:** Fifty ml of the appropriate test or control solution were placed into each of three

replicate flasks (3 per treatment level and the control).

A sample of a *S. capricornutum* culture was diluted with AAP medium, and the density was determined. An inoculum of cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.241 ml per flask. The flasks were randomly repositioned each working day to minimize spatial differences in the incubator. Cell counts were performed using an electronic particle counter on test days 3, 4, and 5.

The pH was measured at test initiation and termination. Temperature was monitored manually daily and continuously with a recording device.

Samples were collected at test initiation for analysis of the test material by high performance liquid chromatography. The samples were shipped on ice to the study sponsor for analysis.

E. **Statistics:** All calculations were based on initial measured concentrations. The EC values and associated 95% confidence intervals (C.I.) were computed using weighted least squares non-linear regression of the cell counts (expressed as inhibition compared to the medium control) at each concentration against the log of the test concentrations. The no-observed-effect concentration (NOEC) was estimated using analysis of variance (ANOVA) and Dunnett's test. The level of significance was $p \leq 0.05$.

12. **REPORTED RESULTS:** Measured concentrations ranged between 86 and 88% of nominal. The initial measured concentrations were 1.10, 2.17, 4.38, 8.55, and 17.4 µg/l (Table 3, attached).

Cell counts and percent inhibition for each concentration after five days are given in Tables 4 and 5 (attached). Exposure to increasing amounts of bromacil had an increasingly inhibitory effect on the algae. Five-day responses ranged from 15.2 to 92.1% inhibition.

The five-day EC_{25} was 5.80 µg/l (95% C.I.= 4.64-7.26 µg/l) and the five-day EC_{50} was 8.44 µg/l (95% C.I.= 7.27-9.80 µg/l). The NOEC was determined to be 1.10 µg/l.

4

The pH ranged from 7.36 to 7.54 in all test solutions and the control at test initiation. The pH values on day 5 ranged from 7.96 to 8.49.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the study 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.

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:

Cell growth measurements were not taken daily. Measurements were made on days 3, 4, and 5 only.

The results of the daily or continuous temperature measurements were not reported.

B. **Statistical Analysis:** The reviewer determined the EC_{50} using EPA's Toxanal program as 6.8 µg/l (95% C.I.= 5.9-7.8 µg/l). The lowest-observed-effect concentration (LOEC) and NOEC were determined using ~~Dunnett's test.~~ Williams test. ~~The reviewer obtained the same NOEC as the authors'~~ (see attached printouts). Since the reviewer's moving average angle method results are more conservative, they will be reported.

C. **Discussion/Results:** This study is scientifically sound ~~and meets~~ Partially the guideline requirements for a Tier 2 non-target aquatic plant study. Based on initial measured concentrations, the 5-day ~~NOEC,~~ LOEC, and EC_{50} for *S. capricornutum* exposed to bromacil technical were 1.1, ~~2.2,~~ and 6.8 µg/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Core.~~ Supplemental

(2) **Rationale:** N/A.

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

15. **COMPLETION OF ONE-LINER:** Yes, 1-12-93.

RIN 6995-94

EEB BROMACIL REVIEW

Page____ is not included in this copy.

Pages 6 through 9 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

V FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

MOSSLER BROMACIL SELENASTRUM CAPRICORNUTUM 2-11-93

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
17.4	100	92	92	0
8.55	100	55	55	0
4.38	100	25	25	0
2.17	100	22	22	0
1.1	100	15	15	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7.678564

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	3.101041E-02	6.757993	5.893996	7.797334

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.6408891	8.342733	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 = 1.875265
95 PERCENT CONFIDENCE LIMITS = .3740113 AND 3.376519

LC50 = 5.946965
95 PERCENT CONFIDENCE LIMITS = 2.364903 AND 29.30402

LC10 = 1.250426
95 PERCENT CONFIDENCE LIMITS = 4.080697E-03 AND 2.8725

****

Ecological Effects Branch One-Liner Data Entry Form

Chemical Bromacil **Shaughnessy No.** 012301 **Pesticide Use** Herbicide

PHYTOTOXICITY AQUATIC SPECIES	% AI	EC_{50} (95%CL)	HRS/ DAYS	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Selenastrum capricornutum	96.5	6.8 mg/l (5.9-7.8)	5-days	1.1 mg/l	1992/1993	425164-01 Core	MPI	MM
2.								
3.								
4.								
5.								

COMMENTS: _____

Selenastrum capricornutum 5 Day Cell Counts for Bromacil x 1000
File: BromaS.cap Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Controls	3	3972.400	4385.400	4212.733
2	1.10 ug/L	3	3154.600	4189.800	3572.467
3	2.17	3	2942.000	3677.600	3281.800
4	4.38	3	2785.400	3646.000	3146.800
5	8.55	3	1780.800	2069.000	1889.267
6	17.4	3	287.100	425.000	333.300

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Controls	46076.333	214.654	123.931
2	1.10 ug/L	297749.973	545.665	315.040
3	2.17	137628.840	370.984	214.187
4	4.38	199399.720	446.542	257.811
5	8.55	24574.013	156.761	90.506
6	17.4	6306.790	79.415	45.850

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	29510782.789	5902156.558	49.756
Within (Error)	12	1423471.340	118622.612	
Total	17	30934254.129		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap 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	Controls	4212.733	4212.733		
2	1.10 ug/L	3572.467	3572.467	2.277	
3	2.17	3281.800	3281.800	3.310	*
4	4.38	3146.800	3146.800	3.790	*
5	8.55	1889.267	1889.267	8.262	*
6	17.4	333.300	333.300	13.795	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap 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	Controls	3			
2	1.10 ug/L	3	703.037	16.7	640.267
3	2.17	3	703.037	16.7	930.933
4	4.38	3	703.037	16.7	1065.933
5	8.55	3	703.037	16.7	2323.467
6	17.4	3	703.037	16.7	3879.433

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	29510782.789	5902156.558	49.756
Within (Error)	12	1423471.340	118622.612	
Total	17	30934254.129		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

		TRANSFORMED	MEAN CALCULATED IN

GROUP	IDENTIFICATION	MEAN	ORIGINAL UNITS	T STAT	SIG
1	Controls	4212.733	4212.733		
2	1.10 ug/L	3572.467	3572.467	2.277	
3	2.17	3281.800	3281.800	3.310	*
4	4.38	3146.800	3146.800	3.790	*
5	8.55	1889.267	1889.267	8.262	*
6	17.4	333.300	333.300	13.795	*

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=12,5)

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap Transform: NO TRANSFORMATION

BONFERRONI T-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	Controls	3			
2	1.10 ug/L	3	753.937	17.9	640.267
3	2.17	3	753.937	17.9	930.933
4	4.38	3	753.937	17.9	1065.933
5	8.55	3	753.937	17.9	2323.467
6	17.4	3	753.937	17.9	3879.433

Selenastrum capricornutum 5 Day Cell Counts Bromacil
File: BromaS.cap Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Controls	3	4212.733	4212.733	4212.733
2	1.10 ug/L	3	3572.467	3572.467	3572.467
3	2.17	3	3281.800	3281.800	3281.800
4	4.38	3	3146.800	3146.800	3146.800
5	8.55	3	1889.267	1889.267	1889.267
6	17.4	3	333.300	333.300	333.300

Selenastrum capricornutum 5 Day Cell Counts Bromacil x1000
File: BromaS.cap Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Controls	4212.733				
1.10 ug/L	3572.467	2.277	*	1.78	k= 1, v=12
2.17	3281.800	3.310	*	1.87	k= 2, v=12

4.38	3146.800	3.790	*	1.90	k= 3, v=12
8.55	1889.267	8.262	*	1.92	k= 4, v=12
17.4	333.300	13.795	*	1.93	k= 5, v=12

s = 344.416

Note: df used for table values are approximate when v > 20.