10-14-92

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

OCT 14 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pendimethalin: Review of Tier 2 Plant Studies.

FROM: Douglas Urban, Acting Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

10/13/92

TO: Walter Waldrop, PM 71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

As part of the reregistration process for the herbicide Pendimethalin, American Cyanamid Company has submitted the following Tier 2 terrestrial and aquatic plant studies:

Chetram, R.S. and J.A. Gagne. 1992. A Tier 2 Plant Phytotoxicity Study For Seedling Emergence Using AC 92,533. Laboratory Study No. BL91-453. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. MRID No. 423722-01.

White, T.L. and J.A. Gagne. 1992. A Tier 2 Plant Phytotoxicity Study For Seed Germination Using AC 92,553. Laboratory Study No. BL91-471. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. MRID No. 423722-02.

Canez, V.M. and J.A. Gagne. 1992. A Tier 2 Plant Phytotoxicity Study For Vegetative Vigor Using AC 92,553. Laboratory Study No. BL91-454. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. MRID No. 423722-03.

Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Green Alga, Selenastrum capricornutum. Laboratory Study ID No. B400-32-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. MRID No. 423722-04.

Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Marine Diatom, Skeletonema costatum. Laboratory Study ID No. B400-32-4. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. MRID No. 423722-05.

Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Freshwater Diatom, Navicula pelliculosa. Laboratory Study ID No. B400-32-3. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. MRID No. 423722-06.

Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Blue-green Alga, Anabaena flos-aquae. Laboratory Project ID No. B400-32-2. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. MRID No. 423722-07

All of the terrestrial plant studies (42372201, 42372202, 42372203) were classified as invalid as a non-treatment control (i.e. minus test material and solvent) was not included in the tests.

Three of the aquatic plant studies (42372205, 42372206, 42372207) were classified as invalid as the solvent controls were contaminated with the test material. The fourth aquatic plant study (42372204) was classified as core.

Please find all applicable data requirements for pendimethalin and their statuses in the attached table. If you have any questions, please contact Tracy Perry at 305-6451 or Henry Craven at 305-5320.

Date: 10/07/92
Case No: 819421
Chemical No: 108501

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition[1]	Use Pattern[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?
6 Basic Studies in Bold					
71-1(a) Acute Avian Oral, Quail/Duck	(TGAI)	A,B,C,D	YES	00059739	NO
71-1(b) Acute Avian Oral, Quail/Duck	(TEP)	-	-	-	-
71-2(a) Acute Avian Diet, Quail	(TGAI)	A,B,C,D	YES	00026674	NO
71-2(b) Acute Avian Diet, Duck	(TGAI)	A,B,C,D	YES	00026675	NO
71-3 Wild Mammal Toxicity	(TGAI)	-	-	-	-
71-4(a) Avian Reproduction Quail	(TGAI)	-	-	-	-
71-4(b) Avian Reproduction Duck	(TGAI)	-	-	-	-
71-5(a) Simulated Terrestrial Field Study	(TEP)	-	-	-	-
71-5(b) Actual Terrestrial Field Study	(TEP)	-	-	-	-
72-1(a) Acute Fish Toxicity Bluegill	(TGAI)	A,B,C,D	YES	00106764	NO
72-1(b) Acute Fish Toxicity Bluegill	(TEP)	D	YES	00037927, FAOPEN01	NO
72-1(c) Acute Fish Toxicity Rainbow Trout	(TGAI)	A,B,C,D	YES	00160764	NO
72-1(d) Acute Fish Toxicity Rainbow Trout	(TEP)	D	YES	FAOPEN01, 00037927	NO
72-2(a) Acute Aquatic Invertebrate Toxicity	(TGAI)	A,B,C,D	YES	FAOPEN05	NO
72-2(b) Acute Aquatic Invertebrate Toxicity	(TEP)	D	YES	260404	NO
72-3(a) Acute Estu/Mari Tox Fish	(TGAI)	A,D	YES	FAOPEN02	NO
72-3(b) Acute Estu/Mari Tox Mollusk	(TGAI)	A,D	YES	FAOPEN03	NO
72-3(c) Acute Estu.Mari Tox Shrimp	(TGAI)	A,D	YES	FAOPEN03	NO

* In Bibliographic Citation column indicates study may be upgradeable

Date: 10/07/92
Case No: 819421
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PHASE IV
DATA REQUIREMENTS FOR
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Data Requirements	Composition[1]	Use Pattern[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?
72-3(d) Acute Estu/Mari Tox Fish	(TEP)	A,D	YES	FAOPEN02	NO
72-3(e) Acute Estu/Mari Tox Mollusk	(TEP)	A,D	YES	FAOPEN03	NO
72-3(f) Acute Estu/Mari Tox Shrimp	(TEP)	A,D	YES	FAOPEN04	NO
72-4(a) Early Life-Stage Fish	(TGAI)	-	-	-	-
72-4(b) Live-Cycle Aquatic Invertebrate	(TGAI)	A,D	YES	00100504	NO
72-5 Life-Cycle Fish	(TGAI)	A,D	YES	00037940	NO
72-6 Aquatic Org. Accumulation	(TGAI)	A,D	NO	-	YES
72-7(a) Simulated Aquatic Field Study	(TEP)	-	-	-	-
72-7(b) Actual Aquatic Field Study	(TEP)	D	NO	-	YES[3]
122-1(a) Seed Germ./Seedling Emerg.	(TGAI)	-	-	-	-
122-1(b) Vegetative Vigor	(TGAI)	-	-	-	-
122-2 Aquatic Plant Growth	(TGAI)	-	-	-	-
123-1(a) Seed Germ./Seedling Emerg.	(TGAI)	A,B,C,D	NO	42372201, 42372202	YES
123-1(b) Vegetative Vigor	(TGAI)	A,B,C,D	NO	42372203	YES
123-2 Aquatic Plant Growth	(TGAI)	A,B,C,D	NO	423722-(04-07)	YES[4]
124-1 Terrestrial Field Study	(TEP)	A,B,C,D	NO	-	YES[5]
124-2 Aquatic Field Study	(TEP)	A,B,C,D	NO	-	YES[5]
141-1 Honey Bee Acute Contact	(TGAI)	A,B,C	YES	00099890	NO
141-2 Honey Bee Residue on Foliage	(TEP)	-	-	-	-
141-5 Field Test for Pollinators	(TEP)	-	-	-	-

* In Bibliographic Citation column indicates study may be upgradeable

1.Composition: TGAI = Technical grade of the active ingredient; PAIRA = Pure active ingredient, radiolabeled; TEP = Typical end-use product

2.Use Patterns: A = Terrestrial Food Crop; B = Terrestrial Feed Crop; C = Terrestrial Non-Food Crop; D = Aquatic Food Crop; E = Aquatic Non-Food Outdoor; F = Aquatic Non-Food Industrial; G = Aquatic Non-Food Residential; H = Greenhouse Food Crop; I = Greenhouse Non-Food Crop; J = Forestry; K = Outdoor Residential; L = Indoor Food; M = Indoor Non-Food; N = Indoor Medical; O = Indoor Residential; Z = Use Group for Site 00000

3. THIS STUDY IS REQUIRED IN ORDER TO SUPPORT THE RICE USE.

4. MRID No. 42372204 (SELENASTRUM CAPRICORNUTUM) HAS BEEN CLASSIFIED AS CORE. FOUR ADDITIONAL AQUATIC PLANT STUDIES ARE OUTSTANDING: SKELETONEMA, NAVICULA, ANABAENA, LEMNA.

5. TIER III FIELD TESTING IS RESERVED PENDING RECEIPT AND REVIEW OF TIER II TESTS.

DP Barcode : D180497
PC Code No : 108501
EEB Out :

To: Walter Waldrop
Product Manager 71
Special Review and Reregistration Division (H7508W)

From: Douglas J. Urban, Acting Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 108501
Chemical Name : Pendimethalin
Type Product : Herbicide
Product Name : Prowl
Company Name : American Cyanamid Company
Purpose : Data Review

Action Code : 606 Date Due : 09/18/92
Reviewer : Tracy L. Perry

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)	42372201 42372202	N N
71-4(B)			72-3(E)			123-1(B)	42372203	N
71-5(A)			72-3(F)			123-2	42372204 42372205 42372206 42372207	Y N N N
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but
N= Unacceptable

DP BARCODE: D180497 REREG CASE #

CASE: 819421 DATA PACKAGE RECORD DATE: 07/10/92
SUBMISSION: S421457 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 606 DATA PACKAGE REVIEW
CHEMICALS: 108501 Pendimethalin (ANSI) 100.00 %

ID#: 108501
COMPANY:
PRODUCT MANAGER: 71 WALTER WALDROP 703-308-8062 ROOM: CS1 3B3
PM TEAM REVIEWER: TERRI STOWE 703-308-8043 ROOM: CS1 3D5
RECEIVED DATE: 06/25/92 DUE OUT DATE: 09/23/92

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 180497 EXPEDITE: Y DATE SENT: 07/10/92 DATE RET.: / /
CHEMICAL: 108501 Pendimethalin (ANSI)
DP TYPE: 001 Submission Related Data Package
ADMIN DUE DATE: 09/18/92 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	07/14/92	/ /
BRAN: EEB	07/14/92	/ /
SECT:	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

ATTENTION: THIS DATA COMPLETES THE DATA PACKAGE FOR THE TIER II PHYTOTOXICITY STUDIES

Please review the pendimethalin data for the following Tier II Phytotoxicity studies:

123-1A	Seedling Emergence	42372201
123-1A	Seed Germination	42372202
123-1B	Vegetative Vigor	42372203
123-2	Aquatic Plant Growth	
	Selenastrum	42372204
	Skeletonema	42372205
	Navicula	42372206
	Anabaena	42372207

The other aquatic plant growth study for Duckweed (MRID 42137101) was submitted earlier under bean sheet S409199, D172758. Please send a copy of the review to:

Terri Stowe
SRRD/RB (H7508W)
Crystal Station I

THANK YOU!!!

For the attached reregistration case, please identify all applicable data requirements and note those for which

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DP BARCODE: D180497 REREG CASE #

CASE: 819421 DATA PACKAGE RECORD DATE: 07/10/92
SUBMISSION: S421457 BEAN SHEET

* * * DATA REVIEW INSTRUCTIONS * * *

adaquate data have not been submitted to the Agency.

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

8

DP BARCODE: D180497 REREG CASE #

CASE: 819421 DATA PACKAGE RECORD DATE: 07/10/92
SUBMISSION: S421457 BEAN SHEET

* * * DATA REVIEW INSTRUCTIONS * * *

adaquate data have not been submitted to the Agency.

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

9

Fe

MRID No. 423722-01

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 92,553 (Pendimethalin). Shaughnessey No. 108501.

2. **TEST MATERIAL:** AC 92,553 technical; CAS No. 40487-42-1; Lot No. AC 6539-77A; 92.98% active ingredient; a yellow to orange-brown solid.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Seedling Emergence Phytotoxicity Test - Tier 2. Species Tested: soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn, and onion.

4. **CITATION:** Chetram, R.S. and J.A. Gagne. 1992. A Tier 2 Plant Phytotoxicity Study For Seedling Emergence Using AC 92,533. Laboratory Study No. BL91-453. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 423722-01.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 10/5/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 10/5/92

7. **CONCLUSIONS:** This study is ~~not~~ scientifically sound and does not meet the requirements for a Tier 2 seedling emergence test as a second control was not included in the test.

upgraded to core/Oct. 1993

Percent emergence: Fourteen DAT, the most sensitive species was ryegrass, with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.02, 0.04, 0.03, and 0.08 lb ai/A, respectively.

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Percent survival: At 21 DAT, the most sensitive species was again ryegrass. The NOEC, LOEC, EC_{25}, and EC_{50} values for ryegrass were 0.02, 0.04, 0.06, and 0.15 lb ai/A, respectively.

Phytotoxicity rating: Ryegrass was also the most sensitive species with respect to damage. The NOEC and LOEC were 0.02 and 0.04 lb ai/A, respectively. No EC values were determined from the phytotoxicity data.

Plant height: The most sensitive species was ryegrass, with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.01, 0.02, 0.05, and 0.24 lb ai/A, respectively.

Plant weight: The most sensitive species was again ryegrass, with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.01, 0.02, 0.02, and 0.03 lb ai/A, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by six species from five families (i.e., soybean, lettuce radish, cucumber, cabbage and tomato) and monocotyledon plants were represented by four species from two families (i.e., corn, oat, ryegrass, and onion). Cultivars, seed sources, lot numbers, and germination ratings were provided in the report.

B. **Test System:** Ten seeds of each crop were planted in plastic pots (7.5 x 7.5 x 6.0 cm), filled with sterilized soil (0.6% organic matter, pH 7.6) and perlite. A plexiglass template was used to create planting holes in the soil, thus allowing for uniform planting depth and seed distribution. Soybean, cucumber, oat, and corn were planted at a 2.5 cm depth. The remaining six species were planted at a 1.3 cm depth. A continuation study was required for ryegrass, and the same planting regime was used in this study.

Each treatment replicate was placed on an aluminum tray (6.25 x 31.25 cm). The spray plot was 4.9 ft^2. All applications were performed using a spray booth equipped with a single nozzle. A nozzle height of 10.5

inches and a nozzle pressure of 35 psi were used. The test spray solutions were prepared by dissolving the test substance in a 67% acetone/deionized water solution, and serially diluting to obtain lower application rates. The plants were sprayed at the equivalent of 468 l/ha (50 gpa) of water within 5.5 hours of formulation.

The pots were hand watered for the first two days to allow the test material to move into the seed zone. During the initial watering the pots received approximately 12 and 7 ml of water for the base and continuation studies, respectively. For the remainder of the study, the plants were watered automatically four times a day and a total of 39 ml of water for the base experiment and 31.3 ml for the continuation study were used to irrigate each pot per day.

C. **Dosage:** The test material was applied at the rates of 0.0, 0.063, 0.13, 0.25, 0.5, 1.0, 2.0, and 4.0 lb active ingredient (ai)/A for the base study. For the ryegrass continuation study, the rates used were 0.0, 0.004, 0.01, 0.02, 0.04, and 0.07 lb ai/A.

D. **Design:** Each crop/treatment combination was replicated four times (i.e., 10 seeds/pot, 4 pots/treatment level). After treatment, pots containing lettuce, cabbage, ryegrass, and onion were held in the spray room at 18-26°C for 48 hours to enhance germination. Pots containing ryegrass in the continuation study were held for 48 hours at 20-30°C. All other crops were immediately placed in an on-site greenhouse. Trays were rotated 180° twice weekly to reduce phototropism.

The base study and ryegrass continuation study were completed 21 days after initiation. Seedling emergence was recorded at 10 and 14 days after treatment (DAT). Seedling survival and plant height were recorded 21 DAT. Phytotoxicity ratings were recorded 10, 14, and 21 DAT. Twenty-one DAT, plants within treatment replicates were cut at the soil level and dried in pre-weighed aluminum foil sheets. Plant material was dried at approximately 70°C for the base study and 100°C for the ryegrass continuation study for a minimum of 48 hours.

The phytotoxicity ratings evaluated five observable toxic effects: 0-indicates no effect; 1-indicates slight plant effect; 2-indicates a moderate effect (e.g., mild stunting or chlorosis); 3-indicates a

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severe effect; 4-indicates a total plant effect (very poor vigor); and 5-plant death.

Samples were collected from the base and continuation study spray solutions. The samples were analyzed for pendimethalin by gas chromatography coupled with nitrogen-phosphorous detection.

Temperature, relative humidity, illuminance, and photoperiod during the period of growth were provided in the report.

E. **Statistics:** All data were entered into a Lotus 1-2-3 spreadsheet. The spreadsheet calculated replicate means, treatment means, standard deviations, and analysis of variance tables. Treatment means were used to calculate the percent effect resulting from the treatment. The percent detrimental effect was calculated using the following equation:

$$\% \text{ effect} = \frac{(\text{treatment mean} - \text{control mean})}{\text{control mean}} \times 100$$

A randomized complete block analysis of variance (ANOVA) was performed on treatment level x replicate means. Prior to analysis, phytotoxicity data were expressed as proportions of the maximum rating (5), and transformed by taking the arcsine of the square root. Treatment level means were submitted to a one-tailed Dunnett's multiple comparison test to determine those treatments that differed from control levels. The no-observed-effect concentration (NOEC) was determined as the highest level not statistically different from the controls or the highest treatment concentration exhibiting a detrimental effect less than 25%.

The percent detrimental effect values were input into a probit analysis program. The program ignored positive values and transformed the dose by natural logarithms.

12. **REPORTED RESULTS:** Results of the analytical measurements are presented in Tables I and II (attached). Recovery of base study solutions averaged 91% of nominal. Recovery of continuation study solutions averaged 98% of nominal. Results are presented as nominal application rates.

The NOEC, EC_{25}, and EC_{50} values for seedling emergence, seedling survival, phytotoxicity, plant height, and dry weight are listed in Table XII (attached).

Percent emergence: By 14 DAT, soybean, lettuce, radish, tomato, cucumber, cabbage, oat, and corn had an NOEC equal to the maximum tested rate, 4.0 lb ai/A. Ryegrass and onion showed true rate responses. The NOEC, EC_{25}, and EC_{50} values for ryegrass were 0.02, 0.03, and 0.08 lb ai/A, respectively. These same values for onion were 0.25, 1.3, and 3.5 lb ai/A, respectively.

Percent survival: At 21 DAT, the species tested exhibited a range of responses to the test material. Soybean, cabbage and oat had an NOEC of 4.0 lb ai/A and did not exhibit a rate response. Radish and cucumber had an NOEC of 2.0 lb ai/A, but did not exhibit a rate response. Tomato and corn had an NOEC of 1.0 lb ai/A. The EC_{25} and EC_{50} values for tomato were 1.6 and 3.0 lb ai/A, respectively. The EC_{25} and EC_{50} values for corn were 1.4 and 2.9 lb ai/A, respectively. The NOEC, EC_{25}, and EC_{50} values for ryegrass were 0.02, 0.06, and 0.15 lb ai/A, respectively. Those for onion were 0.25, 0.28, and 0.82 lb ai/A, respectively.

Phytotoxicity rating: The NOECs (in lb ai/A) in order of increasing sensitivity for all the crops tested were:

cucumber (2.0) < soybean (1.0) < radish = tomato (0.5) < cabbage = oat = corn (0.25) < lettuce = onion (0.063) < ryegrass (0.02).

No EC values were determined from the phytotoxicity data.

Plant height: For plant height, EC_{50} values were not determined for soybean, radish, and cucumber since none of the treatment levels caused height reduction greater than or equal to 50%. The response of the ten species tested ranged greatly in terms of plant height (Table XII). The most sensitive species was ryegrass with NOEC, EC_{25}, and EC_{50} values of 0.01, 0.05, and 0.24 lb ai/A, respectively.

Plant dry weight: For plant dry weight, EC_{50} values were not determined for soybean, radish, cucumber, and oat since none of the treatment levels caused weight reduction greater than or equal to 50%. The response of the ten species tested ranged greatly in terms of plant weight (Table XII). The most sensitive species was again ryegrass with NOEC, EC_{25}, and EC_{50} values of 0.01, 0.01, and 0.03 lb ai/A, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No other conclusions other than those stated above were made by the authors.

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc., stated that Good Laboratory Practice (GLP) Standards (40 CFR Part 160) were employed. Statements of Compliance with GLPs and Quality Assurance were provided.

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

A. Test Procedure: The test procedures followed the SEP and Subdivision J guidelines, except for the following:

Only a solvent control was included in the study. A second control (i.e. minus solvent) was not present.

B. Statistical Analysis: Probit analysis was conducted on ryegrass dry weight (the most sensitive species) data to determine the EC values and ANOVA (coupled with Dunnett's test) was used to verify the NOEC and lowest-observed-effect concentration (LOEC). The results are in general agreement with the authors' (see attached printouts). However, the EC_{25} should have been rounded to 0.02 lb ai/A.

C. Discussion/Results: Since measured concentrations were greater than 90% of nominal, the reviewer accepts the nominal rates listed as representative of the rates applied.

Percent emergence: Fourteen DAT, the most sensitive species was ryegrass, with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.02, 0.04, 0.03, and 0.08 lb ai/A, respectively. All EC values listed as ND in Table XII should be considered as >4.0 lb ai/A.

Percent survival: At 21 DAT, the most sensitive species was again ryegrass. Although the two studies failed to produce independent response curves, the EC values determined by the author are considered to be adequate representation of the test material's toxicity to this species. The NOEC, LOEC, EC_{25}, and EC_{50} values for ryegrass were 0.02, 0.04, 0.06, and 0.15 lb ai/A, respectively. All EC values listed as ND in Table XII should be considered as >4.0 lb ai/A.

Phytotoxicity rating: Ryegrass was also the most sensitive species with respect to damage. The NOEC and LOEC were 0.02 and 0.04 lb ai/A, respectively. No EC values were determined from the phytotoxicity data.

Plant height: The most sensitive species was ryegrass, with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.01, 0.02,

0.05, and 0.24 lb ai/A, respectively. All EC values listed as * in Table XII should be considered as >4.0 lb ai/A.

Plant weight: The most sensitive species was again ryegrass, with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.01, 0.02, 0.02, and 0.03 lb ai/A, respectively. All EC values listed as * in Table XII should be considered as >4.0 lb ai/A.

This study is not scientifically sound and does not fulfill the guideline requirements for a Tier 2 seedling emergence study as a second control was not included in the test.

D. **Adequacy of the Study:** Core

(1) **Classification:** ~~Invalid.~~ See Oct 93 Memo

(2) **Rationale:** A second control (i.e. minus solvent) was not included in the test.

(3) **Repairability:** None.

15. **COMPLETION OF ONE-LINER:** N/A.

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Pendimethalin

Page _____ is not included in this copy.

Pages 17 through 18 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

X 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.

SEEDLING EMERGENCE -PENDIMETHALIN - RYEGRASS DRY WGT

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	4	.1230	.0174	14.2
2 0.004 Δ	4	.1270	.0133	10.5
3 0.01	4	.1088	.0162	14.9
4* 0.02	4	.0687	.0145	21.1
5* 0.04	4	.0440	.0114	25.8
6* 0.07	4	.0353	.0113	32.1
7* 0.13	4	.0030	.0048	158.7
8* 0.25	4	.0000	.0000	.0

rate (lb ai/A)

NOEC = 0.01 lb ai/A

LOEC = 0.02 lb ai/A

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Δ dose based on nominal concentration in lb a.i./A

Minumum detectable difference for Dunnett's test = -.021786
This difference corresponds to -17.71 percent of control

Between groups sum of squares = .074072 with 7 degrees of freedom.

Error mean square = .000154 with 24 degrees of freedom.

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**************************************************
*                                                *
* Warning - the test for equality of variances   *
* could not be computed as 1 or more of the      *
* variances is zero.                             *
*                                                *
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SEEDLING EMREGENCE - PENDIMETHALIN - RYEGRASS DRY WGT

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0035	0.0013	0.0061
EC 5.00	0.0065	0.0031	0.0101
EC10.00	0.0090	0.0048	0.0132
EC15.00	0.0113	0.0066	0.0160
EC50.00	0.0293	0.0217	0.0388
EC85.00	0.0760	0.0554	0.1224
EC90.00	0.0952	0.0672	0.1652
EC95.00	0.1329	0.0888	0.2597
EC99.00	0.2485	0.1474	0.6159

$Y = 8.84 + 2.51(x)$

Y = probit % inhibition

X = log (rate)

$EC_{25} = 0.016$ 16 ai/A

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 92,553 (Pendimethalin). Shaughnessey No. 108501.

2. **TEST MATERIAL:** AC 92,553 technical; CAS No. 40487-42-1; Lot No. AC 6539-77A; 92.98% active ingredient; a yellow to orange-brown solid.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Seed Germination Phytotoxicity Test - Tier 2. Species Tested: soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn, and onion.

4. **CITATION:** White, T.L. and J.A. Gagne. 1992. A Tier 2 Plant Phytotoxicity Study For Seed Germination Using AC 92,553. Laboratory Study No. BL91-471. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 423722-02.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 10/5/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 10/9/92

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements for a Tier 2 Seed Germination Phytotoxicity Test as a second control (i.e. minus solvent) was not included in the test. Ryegrass germination was the most sensitive parameter with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.25, 0.50, 0.82, and 3.5 lb ai/A, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by six species from five families (i.e., soybean, lettuce radish, cucumber, cabbage, and tomato) and monocotyledon plants were represented by four species from two families (i.e., corn, oat, ryegrass, and onion). Cultivars, seed sources, lot numbers, and germination ratings were provided in the report.

B. **Test System:** Two circles of blue blotter were placed in the bottom of a glass petri plate. Six milliliters of the test solution were added to each plate of soybean, cucumber, oat, and corn. Five milliliters were added to plates of lettuce, radish, tomato, cabbage, ryegrass, and onion. The test solution was allowed to evaporate and 12 ml of 5% acetone in deionized water were added to the plates containing soybean, cucumber, oat, and corn. Ten ml of 5% acetone in deionized water were added to the plates containing lettuce, radish, tomato, cabbage, ryegrass, and onion. Control plates consisted of 5% acetone in deionized water that contained no test material.

Ten seeds of each crop were added to each petri plate after the test solution was absorbed into the paper. The plates containing crops with the test solution were impartially placed in plastic boxes (12.25 x 9.0 x 4.1 inches) with tight-fitting lids to prevent moisture loss. The petri plates were incubated in the dark at 25 ±1°C, except lettuce, which was incubated at 20 ±1°C, for 6 days.

C. **Dosage:** The test material was applied at the rates of 0.0, 0.063, 0.13, 0.25, 0.5, 1.0, 2.0, 4.0 lb active ingredient (ai)/A. This was accomplished by preparing a stock solution in 100% hexane at a concentration of 24 ppm. Once the acetone-water hydrating solution was added to the plates, 24 ppm corresponded to the highest application rate (i,e., a 2:1 dilution resulting in a 12 ppm solution). Lower rates were prepared in the same manner by serial dilution of the highest stock solution.

D. **Design:** Each crop/treatment combination was replicated four times (i.e., 10 seeds/plate, 4 plates/treatment

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level). After incubation, germinated seeds were removed from the petri plates and radicle length determined. A seed with a radicle length of 5 mm or greater was considered germinated.

Samples of the stock solutions were collected for determination of pendimethalin concentration by gas chromatography coupled with nitrogen-phosphorous detection.

E. **Statistics:** All data were entered into a Lotus 1-2-3 spreadsheet. The spreadsheet calculated replicate means, treatment means, standard deviations, and analysis of variance tables. Treatment means were used to calculate the percent effect resulting from the treatment. The percent effect was calculated using the following equation:

$$\% \text{ effect} = \frac{(\text{treatment mean} - \text{control mean})}{\text{control mean}} \times 100$$

A randomized complete block analysis of variance (ANOVA) was performed on treatment level x replicate means. Treatment level means were submitted to a one-tailed Dunnett's multiple comparison test to determine those treatments that differed from control levels. The no-observed-effect concentration (NOEC) was determined as the highest level not statistically different from the controls or the highest treatment concentration exhibiting a detrimental effect of less than 25%.

The percent detrimental effect values were input into a probit analysis program to determine the EC values. The program ignored positive values and transformed the dose by natural logarithms.

12. **REPORTED RESULTS:** The NOEC, EC_{25}, and EC_{50} values for seed germination are listed in Table VI (attached).

Analysis using gas-liquid chromatography indicated that the test solutions were 82 to 108% of nominal. All statistical analyses are based on nominal concentrations.

No significant difference in germination percentage existed between controls and any treatment concentration for soybean, lettuce, radish, tomato, cucumber, corn, and onion. Oat exhibited a reduction in germination at the two highest did not exhibit a statistically significant response to any rate of the test material. However, there was a biological

response of >25% at 2 lb ai/A, resulting in an NOEC of 1 lb ai/A. Ryegrass exhibited a significant response at 0.5 lb ai/A, resulting in an NOEC of 0.25 lb ai/A. Ryegrass was the most sensitive species in terms of germination, with EC_{25} and EC_{50} values of 0.77 and 3.45 lb ai/A, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions other than those previously stated were made by the authors.

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc., stated that Good Laboratory Practice (GLP) Standards were employed (40 CFR Part 160). Statements of Compliance with GLPs and Quality Assurance were provided.

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

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines except for the following deviation:

A second control (i.e. minus solvent) was not included in the test.

B. **Statistical Analysis:** Probit analysis was conducted on ryegrass reduction in germination percentage (the most sensitive species) data to determine the EC values and ANOVA (coupled with Dunnett's test) was used to verify the NOEC and lowest-observed-effect concentration (LOEC). The reviewer's model determined a higher EC_{50} than the authors'. Results from ANOVA were in agreement with the authors' (see attached printouts).

C. **Discussion/Results:** Since measured recoveries averaged 98% of nominal, the reviewer accepts the nominal concentrations as adequate representatives of actual rates applied. All values listed as ND in Table VI should be considered as >4.0 lb ai/A.

This study is not scientifically sound and does not fulfill the guideline requirements for a Tier 2 Seed Germination Phytotoxicity Test as a control was not included in the test. Ryegrass germination was the most sensitive parameter with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.25, 0.50, 0.82, and 3.5 lb ai/A, respectively.

D. **Adequacy of the Study:**

(1) Classification: Invalid.

(2) **Rationale:** A second control was not included in the test.

(3) **Repairability:** None.

15. **COMPLETION OF ONE-LINER:** N/A.

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Pendimethalin

Page 26 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

X 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.

SEED GERMINATION - PENDIMETHALIN - RYEGRASS

Summary Statistics and ANOVA

Transformation = None

Group (rate (lb ai/A))	n	Mean	s.d.	cv%
1 = control	4	80.0000	.0000	.0
2 0.063[A]	4	75.0000	5.7735	7.7
3 0.13	4	75.0000	5.7735	7.7
4 0.25	4	77.5000	9.5743	12.4
5* 0.5	4	47.5000	22.1736	46.7
6 1	4	75.0000	10.0000	13.3
7* 2	4	60.0000	8.1650	13.6
8* 4	4	22.5000	5.0000	22.2

NOEC = 0.25 lb ai/A

LOEC = 0.50 lb ai/A

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

[A] based on nominal concentration in lb a.i./A.

Minumum detectable difference for Dunnett's test = -17.987125
This difference corresponds to -22.48 percent of control

Between groups sum of squares = 11246.875000 with 7 degrees of freedom.

Error mean square = 105.208333 with 24 degrees of freedom.

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* Warning - the test for equality of variances   *
* could not be computed as 1 or more of the      *
* variances is zero.                             *
*                                                *
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NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

TRACY PERRY PENDIMETHALIN SEED GERMINATION - RYEGRASS

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
4	80	57	71.25	0
2	80	20	25	0
1	80	5	6.25	0
.5	80	32	40	0
.25	80	2	2.5	0
.13	80	5	6.25	0
.063	80	5	6.25	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 [redacted]51

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.1058084	[redacted]75	2.596062	3.298457

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.160399	14.26976	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 = 1.074032
95 PERCENT CONFIDENCE LIMITS =-8.293474E-02 AND 2.230998

LC50 = [redacted]1
95 PERCENT CONFIDENCE LIMITS = .9241979 AND +INFINITY

LC10 = .2298379
95 PERCENT CONFIDENCE LIMITS = 0 AND .8677961

****

$EC_{25} = 0.82$

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 92,553 (Pendimethalin).
Shaughnessey No. 108501.

2. **TEST MATERIAL:** AC 92,553 technical; CAS No. 40487-42-1; Lot No. AC 6539-77A; 92.98% active ingredient; a yellow to orange-brown solid.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Vegetative Vigor Phytotoxicity Test - Tier 2. Species Tested: soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn, and onion.

4. **CITATION:** Canez, V.M. and J.A. Gagne. 1992. A Tier 2 Plant Phytotoxicity Study For Vegetative Vigor Using AC 92,553. Laboratory Study No. BL91-454. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 423722-03.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 10/5/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T Craven
Date: 10/9/92

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 vegetative vigor non-target phytotoxicity test as a second control was not included in the test.

Phytotoxicity rating: The most sensitive species with respect to plant damage were equally lettuce and ryegrass.

The NOEC and LOEC for these two species were 0.063 and 0.13 lb ai/A, respectively.

No EC values were determined from the phytotoxicity data.

Percent survival: Although the species with the lowest NOEC was ryegrass, onion was determined to be the most sensitive species with respect to survival. The NOEC, LOEC, EC_{25}, and EC_{50} for onion were 1.0, 2.0, 1.4, and 4.5 lb ai/A, respectively. All EC values listed as ND in Table VIII should be considered as >4.0 lb ai/A.

Plant height: The most sensitive species with respect to height was ryegrass. The NOEC, LOEC, EC_{25}, and EC_{50} for ryegrass were 0.063, 0.13, 0.10, and 0.64 lb ai/A, respectively. All EC values listed as ND in Table VIII should be considered as >4.0 lb ai/A.

Dry weight: The NOEC for lettuce dry weight was not determined as the lowest rate applied was significantly different from the control. The NOEC for lettuce dry weight was therefore <0.063 lb ai/A.

Excluding lettuce, the most sensitive species with respect to dry weight was ryegrass. The NOEC, LOEC, EC_{25}, and EC_{50} for ryegrass were 0.06, 0.13, 0.035, and 0.21 lb ai/A, respectively. All EC values listed as ND in Table VIII should be considered as >4.0 lb ai/A, except for tomato, in which case the maximum rate of 4.0 lb ai/A serves as a reasonable estimate of the EC_{50}.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by six species from five families (i.e., soybean, lettuce, radish, cucumber, cabbage, and tomato) and monocotyledon plants were represented by four species from two families (i.e., corn, oat, ryegrass, and onion). Cultivars, seed sources, lot numbers, and germination ratings were provided in the report.

B. **Test System:** Seeds of uniform size were planted in plastic pots (7.5 x 7.5 x 6.0 cm), filled with sterilized soil (0.6% organic matter, pH 7.6) and

30

perlite (20%). A plexiglass template was used to create planting holes in the soil, thus allowing for uniform planting depth and seed distribution. Soybean, cucumber, oat, and corn were planted at a 2.5 cm depth. The remaining six species were planted at a 1.3 cm depth. After planting, the pots were placed in a greenhouse and adequately watered for seedling emergence and growth (9-15 days). The seedlings were grown to 1-3 true leaves and then thinned to five plants per pot.

Each treatment replicate was placed on an aluminum tray (6.5 x 31.25 inches). The spray plot was 4.9 ft^2. All applications were performed using a spray booth equipped with a single nozzle. A nozzle height of 10.5 inches and a nozzle pressure of 30 psi were used. The test spray solutions were prepared by dissolving the test substance in a 67% acetone/deionized water solution, and serially diluting to obtain lower application rate solutions. The plants were sprayed at the equivalent of 468 l/ha (50 gpa) of water.

The pots were hand watered for the first two days on an as-needed basis. For the remainder of the study the plants were watered automatically four times a day and a total of 33 ml of water was used to irrigate each pot per day.

C. **Dosage:** The test material was applied at the rates of 0.0, 0.063, 0.13, 0.25, 0.5, 1.0, 2.0, 4.0 lb active ingredient (ai)/A to all species.

D. **Design:** Each crop/treatment combination was replicated four times (i.e., 5 plants/pot, 4 pots/treatment level). After treatment, the pots containing all species were immediately placed in an on-site greenhouse. Trays were rotated 180° twice weekly to reduce phototropism.

The study was completed 21 days after treatment (DAT). Plant height was recorded prior to treatment and 21 DAT. Phytotoxicity ratings were recorded 7, 14, and 21 DAT. Twenty-one DAT, plants within treatment replicates were cut at the soil level and dried in pre-weighed aluminum foil sheets. Plant material was dried at approximately 70°C for a minimum of 48 hours.

The phytotoxicity ratings evaluated five observable toxic effects: 0-indicates no effect; 1-indicates

slight plant effect; 2-indicates a moderate effect (e.g., mild stunting or chlorosis); 3-indicates a severe effect; 4-indicates a total plant effect (very poor vigor); and 5-plant death.

Samples were collected from the spray solutions and analyzed for pendimethalin by gas chromatography coupled with nitrogen-phosphorous detection.

Temperature, relative humidity, illuminance, and photoperiod during the period of growth were provided in the report.

E. **Statistics:** All data were entered into a Lotus 1-2-3 spreadsheet. The spreadsheet calculated replicate means, treatment means, standard deviations, and analysis of variance tables. Treatment means were used to calculate the percent effect resulting from the treatment. The percent effect was calculated using the following equation:

$$\% \text{ effect} = \frac{(\text{treatment mean} - \text{control mean})}{\text{control mean}} \times 100$$

A randomized complete block analysis of variance (ANOVA) was performed on treatment level x replicate means. Prior to analysis, phytotoxicity data were expressed as proportions of the maximum rating (5), and transformed by taking the arcsine of the square root. Treatment level means were submitted to a one-tailed Dunnett's comparison test to determine those treatments that differed from control levels. The no-observed-effect concentration (NOEC) was determined as the highest level not statistically different from the controls or the highest treatment concentration exhibiting a detrimental effect less than 25%.

The percent detrimental effect values were input into a probit analysis program. The program ignored positive values and transformed the dose by natural logarithms.

12. **REPORTED RESULTS:** Results of the analytical measurements are presented in Table I (attached). Recovery of spray solutions averaged 90% of nominal. Results are presented as nominal application rates.

The NOEC, EC_{25}, and EC_{50} for phytotoxicity, seedling survival, plant height, and dry weight are listed in Table VIII (attached).

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Phytotoxicity rating: By 10 DAT, all crops except radish, cabbage, and onion exhibited significant phytotoxic symptoms. Cabbage did not respond to application of the test material, resulting in an NOEC of 4 lb ai/A. The remaining species varied in their symptom expression. Phytotoxicity on radish, cucumber, ryegrass, corn, and onion increased in severity from 7 to 14 DAT. The severity of phytotoxicity to soybean decreased as the study progressed. The NOECs for each species in order of increasing sensitivity (in lb ai/A) are:

cabbage (4.0) < tomato (2.0) < cucumber (1.0) < corn = onion (0.5) < radish = oat (0.25) < soybean (0.13) < lettuce = ryegrass (0.063).

No EC values were determined from the phytotoxicity data.

Percent survival: Ryegrass exhibited a biologically significant response at 0.5 lb ai/A, resulting in an NOEC of 0.25 lb ai/A. Onion exhibited a significant response at 1.0 lb ai/A, resulting in an NOEC of 0.5 lb ai/A. All other crops tested had an NOEC value of 4.0 lb ai/A. Onion was the only crop to exhibit a rate response, therefore, it was the only crop for which EC values were calculated. The EC_{25} and EC_{50} for onion are 1.4 and 4.5 lb ai/A, respectively.

Plant height: All crops tested, except radish, showed a response to the test material. Crops listed in order of increasing sensitivity based on NOEC for plant height (in lb ai/A) are:

radish (4.0) < lettuce (2.0) < corn = onion (0.5) < oat (0.25) < soybean = tomato = cucumber = cabbage (0.13) < ryegrass (0.063).

Soybean, ryegrass, and onion showed a rate response sufficient to calculate both EC_{25} and EC_{50} values. The rate response of tomato, oat, and corn were sufficient to calculate only EC_{25} values. Due to a lack of response ≥25%, EC values for lettuce, radish, cucumber, and cabbage were not determined.

The EC_{25} and EC_{50} values for soybean, ryegrass, and onion are: 0.48 and 2.1 lb ai/A, 0.1 and 0.63 lb ai/A, and 0.67 and 1.5 lb ai/A, respectively. The EC_{25} values for tomato, oat, and corn are 3.5, 1.0, and 1.6 lb ai/A, respectively.

Plant dry weight: All crops, except radish and cucumber, showed a significant reduction in dry weight. Crops listed

in order of increasing sensitivity to the test material based on NOEC values (in lb ai/A) are:

radish = cucumber (4.0) < cabbage = corn (2.0) < oat = onion (0.5) < soybean = tomato (0.13) < lettuce = ryegrass (0.063).

Soybean, lettuce, oat, ryegrass, and onion showed a rate response sufficient to determine both EC_{25} and EC_{50} values. Tomato, cabbage, and corn showed a rate response sufficient to determine only the EC_{25}. No EC values were determined for radish and cucumber. The EC_{25} and EC_{50} values (lb ai/A) for soybean, lettuce, oat, ryegrass, and onion are: 0.27 and 2.0 lb ai/A, 0.1 and 0.13 lb ai/A, 0.78 and 2.3 lb ai/A, 0.034 and 0.21 lb ai/A, and 0.56 and 1.1 lb ai/A, respectively. The EC_{25} values for tomato, cabbage, and corn are 0.5, 4.8, and 2.8 lb ai/A, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No other conclusions other than those previously mentioned were presented by the authors.

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc., stated that Good Laboratory Practice (GLP) Standards (40 CFR Part 160) were employed. Statements of Compliance with GLPs and Quality Assurance were provided.

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

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines, except for the following:

Only a solvent control was included in the study. A second control was not present.

B. **Statistical Analysis:** Probit analysis was conducted on ryegrass dry weight (the most sensitive species) data to determine the EC values and ANOVA (coupled with William's test) was used to verify the NOEC and lowest-observed-effect concentration (LOEC). The results from the probit analysis differed slightly from those of the authors' and will be taken to be the correct values. The results of the ANOVA are in agreement with the authors' (see attached printouts).

C. **Discussion/Results:** Since measured concentrations were greater than 90% of nominal, the reviewer accepts the nominal rates listed as representative of the rates applied.

Phytotoxicity rating: The most sensitive species with respect to plant damage were equally lettuce and ryegrass. The NOEC and LOEC for these two species were 0.063 and 0.13 lb ai/A, respectively.

No EC values were determined from the phytotoxicity data.

Percent survival: Although the species with the lowest NOEC was ryegrass, onion was determined to be the most sensitive species with respect to survival. The NOEC, LOEC, EC_{25}, and EC_{50} for onion were 1.0, 2.0, 1.4, and 4.5 lb ai/A, respectively. All EC values listed as ND in Table VIII should be considered as >4.0 lb ai/A.

Plant height: The most sensitive species with respect to height was ryegrass. The NOEC, LOEC, EC_{25}, and EC_{50} for ryegrass were 0.063, 0.13, 0.10, and 0.64 lb ai/A, respectively. All EC values listed as ND in Table VIII should be considered as >4.0 lb ai/A.

Dry weight: The NOEC for lettuce dry weight was not determined as the lowest rate applied was significantly different from the control. The NOEC for lettuce dry weight was therefore <0.063 lb ai/A.

Excluding lettuce, the most sensitive species with respect to dry weight was ryegrass. The NOEC, LOEC, EC_{25}, and EC_{50} for ryegrass were 0.06, 0.13, 0.035, and 0.21 lb ai/A, respectively. All EC values listed as ND in Table VIII should be considered as >4.0 lb ai/A, except for tomato, in which case the maximum rate of 4.0 lb ai/A serves as a reasonable estimate of the EC_{50}.

This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 vegetative vigor non-target phytotoxicity test as a control was not included in the study. In addition, the NOEL for lettuce dry weight was not determined.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** A second control (i.e. minus solvent) was not included in the study.

(3) **Repairability:** None.

35

Pendimethalin

Page _____ is not included in this copy.

Pages 36 through 37 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

X 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.

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cV%
rate (lb ai/A)				
1 = control	4	.2610	.0754	28.9
2 0.063[Δ]	4	.2203	.0549	24.9
3* 0.13	4	.1540	.0500	32.5
4* 0.25	4	.0760	.0251	33.0
5* 0.5	4	.0883	.0851	96.4
6* 1	4	.0413	.0035	8.5
7* 2	4	.0623	.0541	87.0
8* 4	4	.0648	.0239	37.0

NOEC = 0.063 lb ai/A
LOEC = 0.13 lb ai/A

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

[Δ] dose based on nominal concentration in lb a.i./A.

Minumum detectable difference for Dunnett's test = -.093190
This difference corresponds to -35.70 percent of control

Between groups sum of squares = .186451 with 7 degrees of freedom.

Error mean square = .002824 with 24 degrees of freedom.

Bartlett's test p-value for equality of variances = .009

****
* *
* Warning - the test for equality of variances *
* is significant (p less than 0.01). The *
* results of this analysis should be inter- *
* preted with caution. *
* *
****

TRACY PERRY PENDIMETHALIN VEGETATIVE VIGOR - RYEGRASS DRY WEIGHT

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
4	100	75	75	0
2	100	76	76	0
1	100	84	84	0
.5	100	66	66	0
.25	100	71	71	0
.13	100	41	41	0
.063	100	16	16	0

THE BINOMIAL TEST SHOWS THAT .13 AND .25 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 .1576265

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	3.465514E-02		.1933704	.1599874

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.6333803	9.185351	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 = .8593384

95 PERCENT CONFIDENCE LIMITS = .1754323 AND 1.543245

LC50 = .215477

95 PERCENT CONFIDENCE LIMITS = 6.537204E-03 AND .647856 $EC_{25} = 0.035$

LC10 = 7.170012E-03

95 PERCENT CONFIDENCE LIMITS = 7.04391E-10 AND 5.210195E-02

****

Pendimethalin: vegetative vigor - lettuce dry weight
File: pendlet.wt Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0.0 lb ai/A	4	0.384	0.534	0.468
2	0.063	4	0.372	0.437	0.392
3	0.13	4	0.299	0.349	0.322
4	0.25	4	0.266	0.327	0.296
5	0.5	4	0.279	0.325	0.294
6	1.0	4	0.217	0.270	0.244
7	2.0	4	0.167	0.262	0.212
8	4.0	4	0.138	0.237	0.188

Pendimethalin: vegetative vigor - lettuce dry weight
File: pendlet.wt Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0.0 lb ai/A	0.004	0.062	0.031
2	0.063	0.001	0.030	0.015
3	0.13	0.000	0.021	0.010
4	0.25	0.001	0.030	0.015
5	0.5	0.000	0.022	0.011
6	1.0	0.000	0.022	0.011
7	2.0	0.002	0.045	0.023
8	4.0	0.002	0.046	0.023

Pendimethalin: vegetative vigor - lettuce dry weight
File: pendlet.wt Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0.0 lb ai/A	4	0.468	0.468	0.468
2	0.063	4	0.392	0.392	0.392
3	0.13	4	0.322	0.322	0.322
4	0.25	4	0.296	0.296	0.296
5	0.5	4	0.294	0.294	0.294
6	1.0	4	0.244	0.244	0.244
7	2.0	4	0.212	0.212	0.212
8	4.0	4	0.188	0.188	0.188

Pendimethalin: vegetative vigor - lettuce dry weight
File: pendlet.wt Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0.0 lb ai/A	0.468				
0.063	0.392	2.873	*	1.71	k= 1, v=24
0.13	0.322	5.499	*	1.79	k= 2, v=24
0.25	0.296	6.473	*	1.82	k= 3, v=24
0.5	0.294	6.577	*	1.83	k= 4, v=24
1.0	0.244	8.466	*	1.84	k= 5, v=24
2.0	0.212	9.685	*	1.84	k= 6, v=24
4.0	0.188	10.564	*	1.85	k= 7, v=24

s = 0.037
Note: df used for table values are approximate when v > 20.

TRACY PERRY PENDIMETHALIN VEGETATIVE VIGOR - LETTUCE DRY WEIGHT

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
4	100	60	60.00001	0
2	100	55	55	0
1	100	48	48	0
.5	100	37	37	0
.25	100	37	37	0
.13	100	31	31	0
.063	100	16	16	0

THE BINOMIAL TEST SHOWS THAT 1 AND 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 1.218775

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.3617362	1.353608	.7357081	2.369836

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.0711227	1	.6118371

SLOPE = .6146216

95 PERCENT CONFIDENCE LIMITS = .4507092 AND .7785341

LC50 = 1.31076

95 PERCENT CONFIDENCE LIMITS = .8940348 AND 2.193987

LC10 = 1.125153E-02

95 PERCENT CONFIDENCE LIMITS = 2.570146E-03 AND 2.713806E-02

****

EC_{25} = 0.10

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 92,553 (Pendimethalin). Shaughnessey No. 108501.

2. **TEST MATERIAL:** AC 92,553 technical; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; CAS No. 40487-42-1; Lot No. AC 6539-77A; 92.98% active ingredient; a yellow to orange-brown solid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Selenastrum capricornutum*.

4. **CITATION:** Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Green Alga, *Selenastrum capricornutum*. Laboratory Study ID No. B400-32-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 423722-04.

5. **REVIEWED BY:**

Donn G. Shilling, Ph.D.
Agronomy Dept.
University of Florida
Gainesville, FL

Signature: Donn G. Shilling
Date: 6/11/92

6. **APPROVED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 6/11/92

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

Signature: Henry T. Craven
Date: 10/9/92

Tracy L. Perry 9/23/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 120-hour NOEC, LOEC, and EC_{50} for *S. capricornutum* exposed to AC 92,553 were 3.0, 4.8, and 5.4 µg ai/l, respectively.

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

9. **BACKGROUND:**

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, Texas. Stock cultures were maintained in algal assay procedure nutrient medium (AAP) under 4306 lux continuous illumination, and a temperature of 24 ±2°C. The cultures were continuously shaken at 100 oscillations per minute. Transfers were made to maintain logarithmic growth. The culture used as inoculum in this test had been transferred to fresh medium 7 days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods 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 stock cultures, 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.

A 1 mg active ingredient (ai)/ml stock was prepared by dissolving 26.9 mg of the test material in N,N-dimethylformamide (DMF), and diluting this to 25 ml with DMF. Other stock solutions (10, 31.25, 62.5, 125[1], 250, and 500 µg ai/ml in DMF) were prepared by serial dilution. Test solutions were prepared by adding appropriate amounts of the stock to nutrient medium.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 2.0, 6.25, 12.5, 25.0, 50.0, and 100 µg ai/l and a medium and solvent control (0.2 ml DMF/l of medium) were selected for the definitive test.

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[1]The authors reported that the other stock solutions were 10.0, 31.25, 62.5, 12.5, 25.0, and 50.0 µg a.i./l. The reviewer feels that the last three concentrations reported are typographical errors and that the actual values are those reported in the body of the text.

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D. **Test Design:** Fifty ml of the appropriate test or control solution were placed into each of three replicate 250-ml flasks (3 per treatment level and the control).

An inoculum of cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.185 ml per flask. The flasks were continuously shaken at 100 oscillations per minute and randomly repositioned each working day to minimize spatial differences in the incubator. Cell counts were performed using a Coulter Counter on test days 3, 4, and 5. Samples ranging from 0.1 to 2.0 ml, depending on the expected population density, were removed from each flask. Three counts per replicate were used on each counting day.

The pH was measured at test initiation (initial test solutions) and termination (replicates combined). Temperature was recorded manually daily and continuously with a recording device.

Samples were taken at test initiation (initial solutions) and termination (replicates combined) for analysis of the test material by gas chromatography. Samples taken at termination were removed from the supernatant of the solutions after centrifuging (3500 rpm) for four minutes to remove the algae.

E. **Statistics:** The data analysis was based on mean measured concentrations of AC 92,553. 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 pooled 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:** The measured concentrations ranged from 59 to 85% of nominal on day 0 and from 10 to 41% of nominal at test termination (Table 3, attached). The mean measured concentrations were 0.795, 3.02, 4.85, 13.4, 26.2, and 51.7 µg ai/l.

Cell counts and percent inhibition for each concentration after five days are given in Tables 4 and 5 (attached). One replicate of the 13.4 µg ai/l treatment had very low cell counts relative to the other replicates in this treatment.

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The cell count value on day five for this replicate was considered as an outlier value and not included in the data analysis. Effects of the test substance on *Selenastrum capricornutum* ranged from 11.3 to 99.9% inhibition.

The EC_{50} was determined to be 6.7 µg ai/l with a 95% C.I. of 5.1 to 8.8 µg ai/l. The NOEC was determined to be 3.0 µg ai/l.

The pH ranged from 7.45 to 7.50 in all test solutions and the controls at test initiation. The pH values on day 5 ranged from 7.82 to 8.53.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

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 and continuous temperature measurements were not reported.

B. **Statistical Analysis:** The reviewer determined the EC_{50} and lowest-observed-effect concentration (LOEC) and NOEC using EPA's probit and Dunnett's test programs, respectively. The EC_{50} determined by the reviewer was lower than that determined by the authors, and will therefore be taken to be the actual value. This value was 5.4 µg ai/l. The LOEC and NOEC were determined to be 4.85 and 3.02 µg ai/l, respectively (see attached printouts).

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 120-hour EC_{50}, LOEC, and NOEC were 5.4, 4.85, and 3.02 µg ai/l, respectively.

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D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 7/27/92.

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Pendimethalin

Page _____ is not included in this copy.

Pages 48 through 51 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

X 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.

SELENASTRUM - PENDIMETHALIN - CELL NUMBER

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper Limits
EC 1.00	0.4882	0.0116	1.4131
EC 5.00	0.9885	0.0611	2.2962
EC10.00	1.4399	0.1462	3.0149
EC15.00	1.8561	0.2610	3.6580
EC50.00	5.4280	2.3652	10.5921
EC85.00	15.8734	8.5238	77.1195
EC90.00	20.4611	10.5074	135.5213
EC95.00	29.8052	13.9935	319.8699
EC99.00	60.3524	23.0348	1665.0487

SELENASTRUM - PENDIMETHALIN - CELL COUNT

Summary Statistics and ANOVA

Transformation = None

Group (Concentration (mg ai/l Δ))	n	Mean	s.d.	cv%
1 = control	6	4593333.3333	229666.4248	5.0
2 0.795 Δ	3	4073333.3333	415852.5380	10.2
3 3.02	3	3853333.3333	202319.8787	5.3
4* 4.85	3	2641333.3333	1569332.7669	59.4
5* 13.4	2	1106000.0000	31112.6984	2.8
6* 26.2	3	9666.6667	6429.1005	66.5
7* 51.7	3	3333.3333	577.3503	17.3

NOEC = 3.02 mg ai/l Δ

LOEC = 4.85 mg ai/l Δ

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

Δ dose based on measured concentration in ug/l (ppb)

Minumum detectable difference for
t-tests with Bonferroni adjustment = -914686.440276
This difference corresponds to -19.91 percent of control

```
**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares =**************** with 6 degrees of freedom.

Error mean square = **************** with 16 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

```
**************************************************
*                                                *
* Warning - the test for equality of variances   *
* is significant (p less than 0.01). The         *
* results of this analysis should be inter-      *
* preted with caution.                           *
*                                                *
**************************************************
```

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 92,553 (Pendimethalin). Shaughnessey No. 108501.

2. **TEST MATERIAL:** AC 92,553 technical; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; CAS No. 40487-42-1; Lot No. AC 6539-77A; 92.98% active ingredient; a yellow to orange-brown solid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Skeletonema costatum*.

4. **CITATION:** Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Marine Diatom, *Skeletonema costatum*. Laboratory Study ID No. B400-32-4. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 423722-05.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 9/23/92

6. **APPROVED BY:**

Henry T. Craven, M.S.
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 10/9/92

7. **CONCLUSIONS:** This study is ~~not~~ scientifically sound and does ~~not~~ meet the guideline requirements for a Tier 2 non-target aquatic plant study as the solvent control was apparently contaminated. Based on mean measured concentrations, the 120-hour NOEC, LOEC, and EC_{50} were 0.7, 1.5, and 5.2 µg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A. Upgraded to Core Oct. 93

9. **BACKGROUND:**

Bgm

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The diatom used in the test, *Skeletonema costatum*, came from laboratory stock cultures originally obtained from the EPA Environmental Research Laboratory in Gulf Breeze, Florida. Stock cultures were maintained in marine algal assay nutrient medium (MAA) under 4306 lux cool-white illumination (14 hour photoperiod), and a temperature of 20 ±2°C. The cultures were manually shaken once per day. Transfers were made to maintain logarithmic growth. The culture used as inoculum for the test had been transferred to fresh medium 7 days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods 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 exception that no EDTA was present. The was pH adjusted to 8.1 ±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. The test vessels were shaken and randomly repositioned each working day to minimize spatial differences in the incubator.

A 1 mg active ingredient (ai)/ml stock was prepared by dissolving 10.8 ±0.1 mg of the test material in N,N-dimethylformamide (DMF), and diluting this to 10 ml with DMF. Other stock solutions (0.03125, 0.0625, 0.125, 0.25, and 0.5 mg ai/l in DMF) were prepared by serial dilution. Test solutions were prepared by adding appropriate amounts of the stock to nutrient medium.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 1.25, 2.5, 5, 10, 20, and 40 µg ai/l and a medium and solvent (0.04 ml DMF/l of medium) control were selected for the definitive test.

D. **Test Design:** Fifty ml of the appropriate test or control solution were placed into each of three replicate 250-ml flasks (3 per treatment level and the controls).

55

An inoculum of cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.672 ml per flask. Cell counts were performed using a Coulter Counter on test days 3, 4, and 5. Samples of 2.0 ml were removed from each flask. Three counts per replicate were used on each counting day.

The pH was measured at test initiation (initial test solutions) and termination (replicates combined). Temperature was recorded manually daily and continuously with a recording device.

Samples were taken at test initiation (initial solutions) and termination (replicates combined) for analysis of the test material by gas chromatography. Samples taken at termination were removed from the supernatant of the solutions after centrifuging (3700 rpm) for four minutes to remove the algae.

E. **Statistics:** The data analysis was based on mean measured concentrations of AC 92,553. 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 pooled 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:** The measured concentrations ranged from 82 to 98% of nominal on day 0 and from 8 to 31% of nominal at test termination (Table 3, attached). The solvent control contained 0.377 µg ai/l of the test material on day 0, but none was detected by day 5. The mean measured concentrations were 0.7, 1.5, 2.7, 5.0, 11.3, and 23.2 µg ai/l.

Cell counts and percent inhibition for each concentration after five days are given in Tables 4 & 5 (attached). One replicate of the 1.5 µg ai/l concentration did not grow and this replicate was treated as an outlier. The other two replicates at this concentration and all three replicates at the two higher concentrations grew. Exposure of *Skeletonema costatum* to AC 92,553 resulted in growth inhibition ranging from 1.7 to 95.4%.

The EC_{50} was determined to be 5.0 µg ai/l with a 95% C.I. of 3.4 to 7.5 µg ai/l. The NOEC was determined to be 2.7 µg ai/l.

The pH ranged from 7.63 to 7.69 in all test solutions and the controls at test initiation. The pH values on day 5 ranged from 7.16 to 7.59.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

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 and continuous temperature measurements were not reported.

A 14-hour photoperiod was used rather than the recommended 16-hour photoperiod.

B. **Statistical Analysis:** The reviewer determined the EC_{50} and lowest-observed-effect-concentration (LOEC) and NOEC using EPA's Toxanal and Dunnett's test programs, respectively. The calculated EC_{50} was similar to the authors', but the C.I. was narrower. Therefore, the reviewer's EC_{50} will be taken to be the correct value. The pooled control was not used due to possible contamination in the solvent control. The NOEC and LOEC were determined to be 0.7 and 1.5 µg ai/l, respectively.

C. **Discussion/Results:** This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 non-target aquatic plant study as the solvent control was contaminated. Based on mean measured concentrations, the 120-hour EC_{50}, LOEC, and NOEC were 5.2, 1.5, and 0.7 µg ai/l, respectively.

57

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Solvent control was apparently contaminated.

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

15. **COMPLETION OF ONE-LINER:** Yes, 7/27/92.

58

Pendimethalin

Page _____ is not included in this copy.

Pages 59 through 62 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

X0 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.

Summary Statistics and ANOVA

Transformation = None

Group (concentration (mg ai/l, *))	n	Mean	s.d.	cv%
1 = control	3	216333.3333	37541.0886	17.4
2 0.7	3	208000.0000	4358.8989	2.1
3 1.5	2	154500.0000	71417.7849	46.2
4 2.7	3	177000.0000	52848.8410	29.9
5 5.0	3	118333.3333	94001.7730	79.4
6* 11.3	3	12666.6667	2886.7513	22.8
7* 23.2	3	9666.6667	577.3503	6.0

NOEC = 5.0 lb ai/A
however, >25% inhibition at 1.5 mg ai/l, ∴
NOEC = 0.7 mg ai/l *
LOEC = 1.5 mg ai/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* based on mean measured concentrations.

Minumum detectable difference for
t-tests with Bonferroni adjustment = -109920.068457
This difference corresponds to -50.81 percent of control

```
**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares =********** with 6 degrees of freedom.

Error mean square = ********** with 13 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

```
**************************************************
*                                                *
* Warning - the test for equality of variances   *
* is significant (p less than 0.01). The         *
* results of this analysis should be inter-      *
* preted with caution.                           *
*                                                *
**************************************************
```

SHILLING SELENASTRUM 7/27/92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
23.2	100	95	95	0
11.3	100	94	94	0
5	100	44	44	0
2.7	100	16	16	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 5.429918

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	2.367759E-02	5.170882	4.693052	5.692892

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.8143635	5.987999	2.508223E-03

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

SLOPE = 3.295341
95 PERCENT CONFIDENCE LIMITS = .3215563 AND 6.269126

LC50 = 5.279841
95 PERCENT CONFIDENCE LIMITS = .9037707 AND 15.05212

LC10 = 2.173844
95 PERCENT CONFIDENCE LIMITS = 2.30371E-04 AND 4.160156

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let

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 92,553 (Pendimethalin).
Shaughnessey No. 108501.

2. **TEST MATERIAL:** AC 92,553 technical; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; CAS No. 40487-42-1; Lot No. AC 6539-77A; 92.98% active ingredient; a yellow to orange-brown solid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: Navicula pelliculosa.

4. **CITATION:** Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Freshwater Diatom, Navicula pelliculosa. Laboratory Study ID No. B400-32-3. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 423722-06.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 9/23/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 10/9/92

7. **CONCLUSIONS:** This study is ~~not~~ scientifically sound and does ~~not~~ meet the guideline requirements for a Tier 2 non-target aquatic plant study as the solvent control was contaminated. Based on mean measured concentrations, the 120-hour NOEC, LOEC, and EC_{50} for N. pelliculosa exposed to pendimethalin were 3.2, 6.0, and 5.8 µg ai/l, respectively.

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

9. **BACKGROUND:**

Upgraded to Core Oct. 94
Bgm

LS

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The diatom used in the test, *Navicula pelliculosa*, came from laboratory stock cultures originally obtained from the University of Texas Culture Collection, Austin, Texas. Stock cultures were maintained in algal assay procedure nutrient medium with added silicon (AAP/Si) under continuous 4306 lux illumination, and a temperature of 24 ±2°C. The cultures were continuously shaken at 100 oscillations per minute. Transfers were made to maintain logarithmic growth. The culture used as inoculum for the test had been transferred to fresh medium 7 days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods 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.

An 1 mg active ingredient (ai)/ml stock was prepared by dissolving 10.8 ±0.1 mg of the test material in N,N-dimethylformamide (DMF), and diluting this to 10 ml with DMF. Other stock solutions (0.02, 0.0625, 0.125, 0.250, and 0.5 mg ai/l in DMF) were prepared by serial dilution. Test solutions were prepared by adding appropriate amounts of the stock to nutrient medium.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 2.0, 6.25, 12.5, 25, 50, and 100 µg ai/l and a medium and solvent (0.1 ml DMF/l of medium) control were selected for the definitive test.

D. **Test Design:** Fifty ml of the appropriate test or control solution were placed into each of four replicate 250-ml flasks (4 per treatment level and the controls).

An inoculum of cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask.

W6

The inoculum volume was 0.145 ml per flask. The flasks were continuously shaken at 100 oscillations per minute. Cell counts were performed using a Coulter Counter on test days 3, 4, and 5. Samples from 0.5 to 2.0 ml, depending on expected population density, were removed from each flask. Three counts per replicate were used on each counting day.

The pH was measured at test initiation (initial test solutions) and termination (replicates combined). Temperature was recorded manually daily and continuously with a recording device.

Samples were taken at test initiation (initial solutions) and termination (replicates combined) for analysis of the test material by gas chromatography. Samples taken at termination were removed from the supernatant of the solutions after centrifuging (3700 rpm) for four minutes to remove the algae.

E. **Statistics:** The data analysis was based on mean measured concentrations of AC 92,553. 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 pooled 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:** The measured concentrations ranged from 91 to 108% of nominal on day 0 and from 2 to 22% of nominal at test termination (Table 3, attached). The mean measured concentrations were 1.2, 3.2, 6.0, 12.6, 28.4 and 56.2 µg ai/l.

Cell counts and percent inhibition for each concentration after five days are given in Tables 4 & 5 (attached). One replicate of the 56.2 µg ai/l concentration contained unusually high cell counts, relative to the other replicates. Microscopic examination indicated that this replicate had been contaminated with an unknown alga. Therefore, the data from this replicate was not used in the analysis. Exposure of *Navicula pelliculosa* to the four highest concentrations resulted in increasing inhibition of growth. Exposure to the test material resulted in 16.1% stimulation at the lowest concentration to 99% inhibition at the highest concentration.

The EC_{50} was determined to be 5.8 µg ai/l with a 95% C.I. of 3.2 to 10.6 µg ai/l. The NOEC was determined to be 3.2 µg ai/l.

The pH ranged from 7.51 to 7.75 in all test solutions and the controls at test initiation. The pH values on day 5 ranged from 7.49 to 7.66.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

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 and continuous temperature measurements were not reported.

B. **Statistical Analysis:** The reviewer determined the EC_{50} and lowest-observed-effect-concentration (LOEC) and NOEC using EPA's Toxanal and Dunnett's test programs, respectively. Only the medium control was used in the ANOVA due to possible contamination in the solvent control. The calculated EC_{50} was less conservative than the authors'. The NOEC and LOEC were determined to be 3.2 and 6.0 µg ai/l, respectively (see attached printouts).

C. **Discussion/Results:** This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 non-target aquatic plant study as the solvent control was apparently contaminated. Based on mean measured concentrations, the 120-hour EC_{50}, LOEC, and NOEC for N. pelliculosa exposed to pendimethalin were 5.8, 6.0, and 3.2 µg ai/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The solvent control was apparently contaminated.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 7/27/92.

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Pendimethalin

Page _____ is not included in this copy.

Pages 70 through 73 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

X FIFRA registration data.

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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.

navicula cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
Concentration (µg ai/l *)				
1 = control	4	666000.0000	451940.9991	67.9
2 1.2	4	697750.0000	467815.0454	67.0
3 3.2	4	642250.0000	362243.3574	56.4
4 6.0	4	262000.0000	205320.8871	78.4
5* 12.6	4	77750.0000	9535.0232	12.3
6* 28.4	4	10750.0000	2872.2813	26.7
7* 56.2	3	6000.0000	1000.0000	16.7

NOEC = 6.0 µg ai/l, however 60% inhib. at this conc.
∴
NOEC = 3.2 µg ai/l *
LOEC = 6.0 µg ai/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* - based on mean measured concentrations.

Minumum detectable difference for
t-tests with Bonferroni adjustment = -552745.101456
This difference corresponds to -82.99 percent of control

```
**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares =********** with 6 degrees of freedom.

Error mean square = ********** with 20 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

```
**************************************************
*                                                *
* Warning - the test for equality of variances   *
* is significant (p less than 0.01). The         *
* results of this analysis should be inter-      *
* preted with caution.                           *
*                                                *
**************************************************
```

BEWICK PENDIMETHALIN NAVICULA 7-27-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
28.4	100	98	98	0
12.6	100	87	87	0
6	100	56	56	0
3.2	100	0	0	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 5.72407

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.027635E-02	7.17685	6.570338	7.803204

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.825753	13.49511	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 = 4.385911

95 PERCENT CONFIDENCE LIMITS =-1.54035 AND 10.31217

LC50 = 6.734309

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 3.45724

95 PERCENT CONFIDENCE LIMITS = 0 AND 7.165477

****

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 92,553 (Pendimethalin). Shaughnessey No. 108501.

2. **TEST MATERIAL:** AC 92,553 technical; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; CAS No. 40487-42-1; Lot No. AC 6539-77A; 92.98% active ingredient; a yellow to orange-brown solid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Anabaena flos-aquae*.

4. **CITATION:** Hughes, J.S., M.M. Alexander, and J.D. Wisk. 1992. Effect of AC 92,553 on Growth of the Blue-green Alga, *Anabaena flos-aquae*. Laboratory Project ID No. B400-32-2. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 423722-07

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 9/23/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 10/9/92

7. **CONCLUSIONS:** This study is ~~not~~ scientifically sound and does ~~not~~ meet the guideline requirements for a Tier 2 non-target aquatic plant study as the solvent control was apparently contaminated. Based on mean measured concentrations, the 120-hour NOEC, LOEC, and EC_{50} for *A. flos-aquae* exposed to pendimethalin were 98, 174, and >174 µg ai/l, respectively.

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

9. **BACKGROUND:**

Later upgraded to Core on Oct. 94

BJM

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, *Anabaena flos-aquae*, came from laboratory stock cultures originally obtained from the American Type Culture Collection, Rockville, MD. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP) under 2153 lux continuous illumination and a temperature of 24 ±2°C. The cultures were manually shaken once each working day. Transfers were made to maintain logarithmic growth. The culture used as inoculum in this test had been transferred to fresh medium 7 days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods and autoclaved before use. Test vessels used were 500-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 growing the stock cultures.

A 1 mg active ingredient (ai)/ml stock was prepared by dissolving 26.9 mg of the test material in N,N-dimethylformamide (DMF), and diluting this to 25 ml with DMF. Other stock solutions (62.5, 125, 250, and 500 µg/ml in DMF) were prepared by serial dilution. Test solutions were prepared by adding appropriate amounts of the stock to nutrient medium.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, five nominal concentrations of 17.5, 35, 70, 140, and 280 µg ai/l and a medium and solvent (0.28 ml DMF/l of medium) control were selected for the definitive test.

D. **Test Design:** One-hundred ml of the appropriate test or control solution were placed into each of three replicate 500-ml flasks (3 per treatment level and the control).

A 5-ml aliquot of an *Anabaena flos-aquae* culture was sonicated and diluted with 7 ml of AAP medium. An inoculum of cells calculated to provide 3,000 cells/ml

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was aseptically introduced into each flask. The inoculum volume was 0.311 ml per flask. The flasks were shaken and 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. Samples were removed from each flask and sonicated for approximately 5 minutes. Three counts per replicate were used on each counting day.

The pH was measured at test initiation (initial test solutions) and termination (replicates combined). Temperature was recorded manually daily and continuously with a recording device.

Samples were taken at test initiation (initial solutions) and termination (replicates combined) for analysis of the test material by gas chromatography. Samples taken at termination were removed from the supernatant of the solutions after centrifuging (3700 rpm) for four minutes to remove the algae.

E. **Statistics:** The data analysis was based on mean measured concentrations of AC 92,553. 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 pooled 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:** The measured concentrations ranged from 71 to 83% of nominal on day 0 and from 51 to 60% of nominal at test termination (Table 3, attached). The mean measured concentrations were 11.7, 24.2, 47, 98, and 174 µg ai/l.

Cell counts and percent inhibition for each concentration after five days are given in Tables 4 and 5 (attached). The test material had little effect on *A. flos-aquae* growth. Responses ranged from 4.1 to 19.6% inhibition.

The EC_{50} could not be determined due to the lack of a true rate response and was reported to be greater than 174 µg ai/l. The NOEC was 174 µg ai/l.

The pH ranged from 7.45 to 7.54 in all test solutions and the controls at test initiation. The pH values on day 5 ranged from 7.40 to 7.77.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

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 and continuous temperature measurements were not reported.

B. **Statistical Analysis:** Since none of the concentrations tested inhibited the growth of *A. flos-aquae* greater than 20%, the EC_{50} was determined to be greater than 174 µg ai/l. The lowest-observed-effect concentration (LOEC) and NOEC were determined using EPA's Dunnett's test program. The NOEC and LOEC were 98 and 174 µg ai/l, respectively (see attached printout).

C. **Discussion/Results:** This study is not scientifically sound and does not meets the guideline requirements for a Tier 2 non-target aquatic plant study as the solvent control was apparently contaminated. Based on mean measured concentrations, the 120-hour NOEC, LOEC, and EC_{50} for *A. flos-aquae* exposed to pendimethalin were 98, 174, and >174 µg ai/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Solvent control was apparently contaminated.

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

15. **COMPLETION OF ONE-LINER:** Yes, 7/28/92.

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Pendimethalin

Page _____ is not included in this copy.
Pages 80 through 83 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

X 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.

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
Concentration (µg ai/l)Δ				
1 = control Δ	6	532.6667	60.8035	11.4
2 11.7	3	488.6667	73.0571	15.0
3 24.2	3	454.6667	21.9393	4.8
4 47.0	3	510.6667	56.7568	11.1
5 98.0	3	478.0000	29.5973	6.2
6* 174.0	3	428.0000	28.8444	6.7

NOEC = 98 µg a.i/l

LOEC = 174 µg a.i/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

Δ dose based on measured concentration in µg/l (ppb)

Minumum detectable difference for
t-tests with Bonferroni adjustment = -77.590184
This difference corresponds to -14.57 percent of control

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**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares = 27909.333333 with 5 degrees of freedom.

Error mean square = 2665.422222 with 15 degrees of freedom.

Bartlett's test p-value for equality of variances = .559