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DPBARCODE (RECORD)
125401
SHAUGHNESSY NO

REVIEW NO.

EEB REVIEW

DATE IN: 3-3-92 OUT: AUG 19 1992
ASSIGNED: 3-11-92
CASE # :016703, 037926
SUB. # :S412836, D412861
ID # :279-3053, 3071

DATE OF SUBMISSION 12-28-91

DATE RECEIVED BY EFED 3-10-92

SRRD/RD REQUESTED COMPLETION DATE 7-4-92

EEB ESTIMATED COMPLETION DATE 7-4-92

SRRD/RD **ACTION** CODE/TYPE OF REVIEW 330 NEW USE

MRID #(S) 123-1 42165705 SEED EMERG STUDY

123-1 42165706 VEG VIGOR STUDY

DP TYPE 001

PRODUCT MANAGER, NO. ROBERT TAYLOR 25 JAMES MORRILL

PRODUCT NAME(S) COMMAND HERBICIDE

TYPE PRODUCT

COMPANY NAME FMC

SUBMISSION PURPOSE REVIEW PLANT DATA, USE TO EVALUATE PROPOSED REQUEST TO ADD ~~TOBACCO~~ COTTON USE TO LABEL

COMMON CHEMICAL NAME

REVIEWER: CHARLES LEWIS

ECOLOGICAL EFFECTS BRANCH REVIEW

Command (Clomazone)

100.1 Submission Purpose and Pesticide Use

The registrant has requested the addition of cotton to the Command 4 E (EPA Reg. No. 279-3071) and Command 4 EC (EPA Reg. No. 279-3053) labels.

100.2 Formulation Information

COMMAND 4 E
ACTIVE INGREDIENTS:----------44.4%
Clomazone: 2-(2-Chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone
INERT INGREDIENTS:----------55.6%

COMMAND 4 EC
ACTIVE INGREDIENTS:----------46.7%
Clomazone: 2-(2-Chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone
INERT INGREDIENTS:----------53.3%

Both products contain 4 pounds active ingredient per gallon.

100.3 Application Methods, Directions, Rates

Use rate is 0.5 to 1.25 lb ai/A applied as a preemergent surface applied treatment in conventional tillage production systems or in reduced tillage production systems such as no-till, minimum-till or reduced-till, or as a preplant incorporated treatment, or as a lay-by treatment for the control of annual grass and broadleaf weeds. One application per year, ground equipment only.

See attached label for complete information.

100.4 Target Organism

Broadleaf weeds and grasses.

See attached label for complete list of species.

100.5 Precautionary Labeling

"Do not apply directly to water or wetlands. Do not apply when weather conditions favor drift from the area treated. Do not apply where runoff is likely to occur. Do not contaminate

water by cleaning of equipment or disposal of wastes. Apply this product only as specified on this label.

"SPECIAL PRECAUTION
Off-site movement of spray drift or vapors of Command 4 EC (4 E) herbicide can cause foliar whitening or yellowing of some plants. Prior to making applications, read and strictly follow all precautions and application instructions on this label."

The labels for these two products contain numerous warnings concerning target phytotoxicity and nontarget phytotoxicity. Refer to the label for the complete list.

101 Hazard Assessment

101.1 Discussion

This request is to add cotton to the Command 4 E and 4 EC labels. Rate of application is 0.5 to 1.25 lb ai/A.

101.2 Likelihood of Adverse Effects on Nontarget Organisms

Terrestrial Organisms

Data from previous reviews indicate that clomazone is practically nontoxic to birds on both an acute oral basis and a dietary basis (bobwhite quail and mallard LD50's >2510 mg/kg, LC50's >5620 ppm). The available data on rats suggest that the chemical also has a low mammalian toxicity.
Maximum residues, based on the nomograph of Kenaga and Hoerger (1972), were calculated to be as follows:

Substrate	Residue (ppm)
Short range grass	300.00
Long grass	137.50
Leaves and leafy crops	156.25
Forage	72.50
Pod containing seeds	15.00
Fruit	8.75

These levels are below calculated or laboratory determined toxicity values for mammals and birds.

No data are available on the effects of clomazone on pollinators.

Aquatic Organisms

Clomazone is slightly toxic to freshwater fish, with LC50's of 19 mg/l for rainbow trout and 34 mg/l for bluegill sunfish. A

daphnid study indicated that clomazone is moderately toxic to aquatic invertebrates (LC50 = 5.2 mg/l). The MATC for Daphnia magna was determined to be between 2.2 and 4.38 mg/l. Estimated environmental concentration (EEC) could be 38.13 ppb in a 1 acre pond 6 feet deep 1/. These values are less than the lowest aquatic LC50 and do not exceed the 1/10 LC50 trigger for restricted use classification using the most sensitive test species. On the basis of these figures, the proposed use of clomazone will not result in hazard to aquatic organisms.

1/ 1.25 lb x 10 acres x 5% runoff x 61 ppb = 38.13 ppb

Nontarget Plants

The potential exists for herbicides to move from the site of application through drift, volatilization, and runoff. COMMAND 4 EC and 4 E will be applied by ground equipment only and drift during application is considered to be negligible. The herbicide has been characterized as volatile (vapor pressure 1.44 x 10-4 mm Hg @ 25 C) and soluble (water solubility 1100 ppm).

Assuming a direct application to 6 inches of water at an application rate of 1.25 lb ai/A, the estimated environmental concentration (EEC) could be 0.9 ppm. This EEC does not exceed the EC50 for Selenastrum capricornutum and does not trigger higher tier testing. Clomazone is not expected to adversely effect freshwater green algae at current application rates. Data on an aquatic macrophyte are not available.

Seed germination is not expected to be adversely affected at current use rates based on the results of tested species. Seedling emergence will be adversely affected at rates of 0.006 lb ai/A (lettuce EC25-plant height). Data on vegetative vigor have been submitted but were unacceptable.

Preplant incorporation is expected to reduce the hazard to nontarget terrestrial plants from off-target movement. However, when applied broadcast preemergence or lay-by the potential increases for clomazone to move from the site of application. Should off-target movement occur, nontarget plants would be expected to be adversely affected.

101.3 Endangered Species Considerations

Hazard to endangered or threatened mammalian, avian, and aquatic species resulting from exposure to COMMAND 4 EC and COMMAND 4 E is considered to be minimal.

Exposure of endangered or threatened plants to clomazone would be expected to be hazardous, however, no endangered or threatened plants have been identified with cotton.

101.4 Adequacy of Toxicity Data

The existing data base is adequate to assess the hazard to nontarget organisms.

The following studies have recently been reviewed:

1) Chetram, R.S. 1990. Tier 2 Seed Germination/Seedling Emergence Nontarget Phytotoxicity Study With Clomazone. Laboratory Project No. LR89-41. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by FMC Corporation, Princeton, NJ. EPA MRID No. 415575-01.

Seed germination. The study is scientifically sound and satisfies the guideline requirement for a Tier II seed germination test. Treatment with Clomazone up to an application rate of 1.5 lb ai/A did not have a significant effect on the germination of any tested species.

Seedling emergence. The study does not meet the guideline requirements for a Tier II seedling emergence test. NOEC concentrations for the most sensitive species (lettuce and tomato) and an absence of raw data were the major discrepancies. Refer to the attached DER for a complete explanation.

Plant height appeared to be the most sensitive plant response evaluated. The most sensitive species, lettuce, had an EC25 and EC50 of 0.008 and 0.013 lb ai/A, respectively. The NOEC could not be determined.

2) Forbis, A.D. and J.N. Wirth. 1990. Acute Toxicity of Clomazone to Selenastrum capricornutum Printz. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. ABC Laboratory Report No. 38065. Submitted by FMC Corporation, Princeton, New Jersey. FMC Corporation Study No. A89-2954. EPA MRID 415575-02.

The study is scientifically sound and fulfills the guideline requirement for a Tier II growth and reproduction test using Selenastrum capricornutum. The 5-day EC50 and EC25, based on mean measured concentrations, were 3.5 and 1.6 mg/L, respectively. The NOEC was 0.99 mg/L.

Assuming a direct application to 6 inches of water at an application rate of 1.25 lb ai/A, the concentration could be 0.9 ppm. This concentration does not exceed the EC50 for Selenastrum capricornutum and does not trigger higher tier testing. Clomazone is not expected to adversely effect freshwater green algae at current application rates.

3) Feutz, E. 1991 Evaluating the Effects of Clomazone on the Vegetative Vigor of Nontarget Terrestrial Plants. Laboratory

Project ID No. 39097. Conducted by ABC Laboratories, Inc., Columbia, MO. Submitted by FMC Corporation, Princeton, NJ. EPA No. 421657-06.

The study does not satisfy the guideline requirement for a Tier II vegetative vigor study. The negative and solvent controls were contaminated with the test compound. Refer to the attached DER for a complete explanation.

4) Chetram, R.S. 1991. Tier 2 Seedling Emergence Nontarget Phytotoxicity Study Using Clomazone. Laboratory Report No. LR90-432. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by FMC Corporation, Princeton, NJ. EPA MRID No. 421657-05.

The study is scientifically sound and satisfies the guideline requirement for a Tier II seedling emergence test using lettuce and tomato.

Percent emergence: Lettuce and tomato were not significantly affected in terms of emergence, but phytotoxic symptoms were observed soon after, followed by plant death for lettuce. The NOEC for both species was 0.15 lb ai/A.

Percent survival: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC25, and EC50 values of 0.0056, 0.0167, 0.005, and 0.010 lb ai/A, respectively.

Phytotoxicity rating: Lettuce was the more sensitive of the two species with NOEC and LOEC values of 0.0056 and 0.0167, respectively.

Plant height: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC25, and EC50 values of 0.0056, 0.0167, 0.006, and 0.013 lb ai/A, respectively.

Plant fresh weight: Lettuce was the most sensitive species with NOEC, LOEC, EC25, and EC50 values of 0.0019, 0.0056, 0.002, and 0.006 lb ai/A, respectively.

Plant dry weight: Lettuce was the most sensitive species with NOEC, LOEC, EC25, and EC50 values of 0.0019, 0.0056, 0.002, and 0.005 lb ai/A, respectively.

The following data are outstanding:

123-1 Vegetative Vigor;
123-2 Aquatic Plant Growth - Lemna gibba;
124-1 Terrestrial Field Testing (postponed);
124-2 Aquatic Field Testing (reserved pending results of aquatic plant testing).

01.5 Adequacy of Labeling

The labeling statement concerning exposure to water and wetlands should be changed to the following: "Do not apply directly to water, areas where surface water is present or to intertidal areas below the mean high water mark."

The label should include a statement that will be very clear to the user that movement of this herbicide could occur after application as a result of co-distillation or volatilization and result in nontarget plant damage.

The statements "Do not apply when weather conditions favor drift from the area treated." and "Do not apply where runoff is likely to occur." should be removed from the label since this is not a practical precautionary statement.

103 Conclusions

EEB has reviewed the request to add weed control in cotton to the Command 4 E and Command 4 EC labels.

Mammals, birds, fish, and freshwater green algae are not expected to be adversely impacted by this new use. The hazard to aquatic macrophytes from runoff is not known at this time. Data for Lemna gibba are outstanding.

Data on the potential for Clomazone to move from the site of application through co-distillation or volatilization and adversely affect nontarget plants are not available. However, EEB feels that the potential for this situation to occur without incorporation is plausible based on previous incidents. Consequently, a Tier III study designed to resolve this concern is required. This study will be postponed until the Agency has developed appropriate test protocols for this test.

Endangered or threatened species are not expected to adversely affected by this use.

Charles R. Lewis Charles R Lewis 8/12/92
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Daniel R. Rieder, Section Head Daniel Rieder 8-17-92
Ecological Effects Branch
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Douglas J. Urban, Acting Chief Norman J Cook for 8.19.92
Ecological Effects Branch
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DATA EVALUATION RECORD

1. **CHEMICAL:** Clomazone.
Shaughnessey No. 125401.

2. **TEST MATERIAL:** Clomazone; 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone; CAS No. 81777-89-1; Sample No. E 5380-49; 98.6% purity; a white crystal turned to light brown liquid.

3. **STUDY TYPE:** Non-Target Plants: Seedling Emergence Phytotoxicity Test - Tier 2. Species Tested: Lettuce and Tomato.

4. **CITATION:** Chetram, R.S. 1991. Tier 2 Seedling Emergence Nontarget Phytotoxicity Study Using Clomazone. Laboratory Report No. LR90-432. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by FMC Corporation, Princeton, NJ. EPA MRID No. 421657-05.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
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Signature:

Date: 6/12/92
8/11/92

6. **APPROVED BY:**

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

Signature:

Date: 8-17-92

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

Signature:

Date: 8/18/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 seedling emergence test using non-target plants.

Percent emergence: Lettuce and tomato were not significantly affected in terms of emergence, but phytotoxic symptoms were observed soon after, followed by plant death for lettuce plants. The NOEC for both of these species was 0.15 lb ai/A.

Percent survival: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0056, 0.0167, 0.005, and 0.010 lb ai/A, respectively.

Phytotoxicity rating: Lettuce was the more sensitive of the two species with NOEC and LOEC values of 0.0056 and 0.0167, respectively.

Plant height: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0056, 0.0167, 0.006, and 0.013 lb ai/A, respectively.

Plant fresh weight: Again, lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0019, 0.0056, 0.002, and 0.006 lb ai/A, respectively.

Plant dry weight: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0019, 0.0056, 0.002, and 0.005 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 two species from two families (i.e., lettuce and tomato). Cultivars, seed sources, lot number, 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 7.8) and perlite. A plexiglass template was used to create planting holes in the soil, thus allowing for uniform planting depth and seed distribution. Seeds of both species were planted at a depth of 1.3 cm.

Each treatment replicate was placed on an aluminum tray (6.125 x 31.125 cm). The spray plot was 38.5 in x 20 in (i.e., 5.35 ft^2). All applications were performed with a belt sprayer equipped with a single nozzle. A nozzle height of 12 inches and a nozzle pressure of 40-45 psi were used. The test spray solutions were prepared by dissolving clomazone in a 5% acetone/well 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.

The pots were watered three times a day and a total of 18 ml of water was used to irrigate each pot per day.

C. **Dosage:** Clomazone was applied at the rates of 0.0006, 0.0019, 0.0056, 0.0167, 0.05, and 0.15 lb active ingredient (ai)/A to the soil in which all species were planted. Treatment application rates were adjusted for the percent active ingredient of the test material.

D. **Design:** Each crop/treatment combination was replicated five times (i.e., 10 seeds/pot, 5 pots/treatment level). After treatment, pots containing tomato seed were placed in an on-site greenhouse. Pots containing lettuce seed were placed in a cool room (25°C) for 48 hours to prevent dormancy from the summer heat. After 48 hours, the lettuce pots were placed in the greenhouse. Trays were rotated 180° twice weekly to reduce phototropism.

The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment. Emerged seedlings and phytotoxicity ratings were recorded at 7, 14, 21, and 28 days after treatment. Twenty-eight days after treatment, plant height was recorded and plants in each replicate were cut at the soil level and dried in pre-weighed aluminum sheets at 70°C for a minimum of 48 hours.

The phytotoxicity ratings evaluated four 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; and 4-indicates a total effect or plant death.

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 detrimental 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 one-way analysis of variance (ANOVA) model for data with equal subsamples was used to analyze data from the emergence portion of the study. A one-way analysis of variance model for data with unequal subsamples was used to analyze seedling height data.

The percent effect values were input into a probit analysis program. The program ignored positive values and transformed the dose by natural logarithms. For emergence, the probit is calculated using all data points. For all other parameters, the probit was calculated using replicate means.

12. **REPORTED RESULTS**: Results are summarized in Table 6 (attached).

Percent emergence: At 7 days after application (DAA), lettuce was fully emerged and was not significantly different ($p < 0.05$) from the control. However, by 14 DAA, clomazone had a significant effect at the three highest concentrations. The no-observed-effect concentration (NOEC) for lettuce 14 DAA was therefore 0.0056 lb ai/A.

At 14 DAA, tomato emergence did not differ significantly from the control. The no-observed-effect concentration (NOEC) was therefore $\geq$0.15 lb ai/A.

Percent survival: At 21 and 28 DAA, lettuce was still significantly affected by clomazone at the three highest rates. The NOEC was 0.0056 lb ai/A. Tomato was still unaffected by any tested rate of clomazone.

The EC_{25} and EC_{50} for lettuce survival were 0.005 and 0.010 lb ai/A, respectively.

Phytotoxicity rating: Both lettuce and tomato were significantly affected by some tested rate of clomazone. The NOECs for these two species were 0.0056 and 0.0167 lb ai/A, respectively. No EC values were determined from the phytotoxicity data.

Plant height: Based on 28 day plant heights, lettuce was more sensitive than tomato and both species demonstrated significant height reductions at some rate of clomazone. The NOECs for lettuce and tomato height were 0.0056 and 0.05 lb ai/A, respectively.

Tomato did not exhibit a dose response and EC values were consequently not determined. The EC_{25} and EC_{50} for lettuce height were 0.006 and 0.013 lb ai/A, respectively.

Plant fresh weight: Based on 28 day plant fresh weights, lettuce and tomato demonstrated significant reductions at the four highest and highest test rates of clomazone, respectively. The NOECs for plant fresh weight for lettuce and tomato were 0.0019 and 0.05 lb ai/A, respectively.

Tomato did not exhibit a dose response, therefore, EC values were not determined. The EC_{25} and EC_{50} for lettuce fresh weight were 0.002 and 0.006 lb ai/A, respectively.

Plant dry weight: The results based on dry weights of the plant tissues are the same as those from fresh weight data with two exceptions. The NOEC for tomato dry weight was 0.0167 lb ai/A rather than 0.05 lb ai/A and the EC_{50} for lettuce was 0.005 lb ai/A rather than 0.006 lb ai/A.

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

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc., stated that Good Laboratory Practice (GLP) Standards 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:

It was not stated in the report whether the control pots were sprayed with the appropriate amount of 5% acetone/well water solution.

The rate progression was three-fold rather than the recommended two-fold.

The material was not applied up to the maximum labeled rate.

The test was conducted with only two dicot species rather than 6 dicots and 4 monocots.

B. **Statistical Analysis:** Probit analysis was conducted on lettuce 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 author's (see attached printouts).

C. **Discussion/Results:** The maximum rate tested in this study was 0.15 lb ai/A. However, it was stated in the summary of the report that this study was a supplement to an earlier study (Pan-Ag LR89-41/FMC 164E7189E1) and that the rates were 10-fold lower than the original study in order to reach the NOECs. This probably explains why the test was only conducted with two species as well. However, missing EC values for lettuce and tomato emergence, and tomato survival, height, and fresh and dry weight will have to be obtained from this other report.

Percent emergence: Lettuce and tomato were not significantly affected in terms of emergence, but phytotoxic symptoms were observed soon after, followed by plant death for lettuce plants. The NOEC for both of these species was 0.15 lb ai/A.

Percent survival: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0056, 0.0167, 0.005, and 0.010 lb ai/A, respectively.

Phytotoxicity rating: Lettuce was the more sensitive of the two species with NOEC and LOEC values of 0.0056 and 0.0167, respectively.

Plant height: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0056, 0.0167, 0.006, and 0.013 lb ai/A, respectively.

Plant fresh weight: Again, lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0019, 0.0056, 0.002, and 0.006 lb ai/A, respectively.

Plant dry weight: Lettuce was the more sensitive of the two species with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.0019, 0.0056, 0.002, and 0.005 lb ai/A, respectively.

Pending the retrieval and assimilation of the data from the previous study to determine the missing EC values,

this study is scientifically sound and meets the guideline requirements for a Tier 2 seedling emergence test using non-target plants.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

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

Table 6 Statistical no-effect concentration (NOEC) (lb ai/A), EC_{25} and EC_{50} values (lb ai/A) for parameters measured during a nontarget plant study with clomazone at 28 days after treatment (Seedling emergence NOEC - 7 days for lettuce and 14 days for tomato).

Crop	Seedling Emergence			Seedling Survival			Phytotoxicity[y]	
	NOEC[x]	EC_{25}	EC_{50}	NOEC	EC_{25}	EC_{50}	NOEC	Mean Rating
Lettuce	> 0.15*	ND[z]	ND	0.0056	0.005	0.010	0.0056	0.0
Tomato	> 0.15*	ND	ND	> 0.15*	ND	ND	0.0167	0.1

Crop	Plant Height			Fresh Weight			Dry Weight		
	NOEC	EC_{25}	EC_{50}	NOEC	EC_{25}	EC_{50}	NOEC	EC_{25}	EC_{50}
Lettuce	0.0056	0.006	0.013	0.0019	0.002	0.006	0.0019	0.002	0.005
Tomato	0.05	ND	ND	0.05	ND	ND	0.0167	ND	ND

* Highest treatment concentration which was not statistically different from the control ($p < 0.05$).

y EC_{25} and EC_{50} values are not normally determined for mean phytotoxicity rating.

z ND = Not determined. If a dose response was not evident or the highest treatment concentration tested did not result in a significant effect, EC_{25} and EC_{50} values could not be determined.

x The highest treatment rate in this study was 0.15 lb ai/A.

lettuce dry weight

Summary Statistics and ANOVA

Transformation = None

Group (rate (lb ai/A*))	n	Mean	s.d.	cv%
1 = control	5	71.0000	15.7003	22.1
2 0.0006	5	59.4000	4.9800	8.4
3 0.0019	5	61.6000	10.2372	16.6
4*0.0056	5	43.8000	11.3446	25.9
5*0.0167	5	4.0000	2.3452	58.6
6*0.05	5	1.6000	2.6077	163.0
7*0.15	5	.0000	.0000	.0

NOEC = 0.0019 lb ai/A*
LOEC = 0.0056 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

* based on nominal ~~am~~ rate of ai

Minumum detectable difference for Dunnett's test = -13.100422
This difference corresponds to -18.45 percent of control

Between groups sum of squares = 29880.342857 with 6 degrees of freedom.

Error mean square = 73.871429 with 28 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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lettuce dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	95% Confidence Lower	95% Confidence Upper
EC 1.00	0.0007	0.0000	0.0016
EC 5.00	0.0013	0.0002	0.0025
EC10.00	0.0018	0.0004	0.0033
EC15.00	0.0022	0.0006	0.0039
EC50.00	0.0060	0.0032	0.0103
EC85.00	0.0158	0.0094	0.0521
EC90.00	0.0200	0.0113	0.0815
EC95.00	0.0281	0.0147	0.1613
EC99.00	0.0534	0.0234	0.5971

$y = 10.43 + 2.44(x)$

y = probit % inhibition

$x = \log(\text{rate})$

$EC_{25} = 0.00316$ ae/A

DATA EVALUATION RECORD

1. **CHEMICAL:** Clomazone.
Shaughnessey No. 125401.

2. **TEST MATERIAL:** Clomazone; 2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone; Lot No. E5380-49; 99.7% purity; a white crystalline solid.

3. **STUDY TYPE:** Non-Target Plants: Vegetative Vigor Nontarget Phytotoxicity Study - Tier 2. Species Tested: Ryegrass, Corn, Oat, Onion, Soybean, Lettuce, Carrot, Tomato, Cucumber, Cabbage.

4. **CITATION:** Feutz, E. 1991. Evaluating the Effects of Clomazone on the Vegetative Vigor of Non-Target Terrestrial Plants. Laboratory Project ID No. 39097. Conducted by ABC Laboratories, Inc., Columbia, MO. Submitted by FMC Corporation, Princeton, NJ. EPA MRID No. 421657-06.

5. **REVIEWED BY:**

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Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]
Date: [signature] 8/11/92

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 6/15/92

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

Signature: [signature] 8-17-92
Date: 8/18/92

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the requirements for a Tier 2 vegetative vigor test using non-target plants. Because the negative and solvent controls were contaminated with the test compound, a valid reference to compare plant damage against was not included in the study.

Phytotoxicity: Onion and tomato were equally the most sensitive species in terms of phytotoxicity. The NOEC and

7

LOEC would be <0.007 and 0.007 lb ai/A for these two species, respectively.

Shoot Length: Onion was the most sensitive species with respect to shoot length. When treatment means were compared to negative control means, the NOEC, LOEC, EC_{25}, and EC_{50} values were 0.022, 0.065, 0.043, and 0.13 lb ai/A, respectively.

Shoot Dry Weight: Onion was again the most sensitive species with respect to shoot dry weight. When treatment means were compared to negative control means, the NOEC, LOEC, EC_{25}, and EC_{50} values were 0.022, 0.065, 0.014, and 0.05 lb ai/A, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

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

B. **Test System:** Seeds of each crop were planted in plastic pots (7.5 inches in diameter and 5.75 inches deep for corn, soybean, and cucumber and 5.5 inches in diameter and 5.25 inches deep for the remaining species) filled 1 inch from the top with a sandy loam soil (pH 7.6, 0.7% organic matter). Plants were watered daily and kept in a greenhouse with a 16-hour supplemented light photoperiod. The plant species were allowed to grow for 11-25 days before treatment to allow each species to attain the 3 true leaf stage and were thinned to a uniform stand of 5 plants per pot.

All applications were performed using a track sprayer equipped with a single nozzle. A nozzle pressure of 35 psi was used. The highest test concentration was prepared by dissolving clomazone in a 10% acetone/deionized water solution that contained 0.25% X-77 as a surfactant. Lower test concentrations were

prepared by serial dilution of the highest concentration with the 10% acetone/deionized water/0.25% X-77 solution. The plants were sprayed at the equivalent of 46 gpa of water.

The pots were watered with deionized water on an as-needed basis. The average daily amount of water used throughout the study was 87 ml/pot. A weak solution of 20-20-20 fertilizer was applied two times during the course of the study. All irrigation was conducted in a manner that prevented rinsing of the foliage.

C. **Dosage:** Clomazone was applied at rates of 0.007, 0.022, 0.065, 0.19, 0.58, and 1.7 lb active ingredient (ai)/A to all plant species.

D. **Design:** Each crop/treatment combination was replicated four times (i.e., 5 plants/pot, 4 pots/treatment level). After treatment, the pots were randomized within species in an on-site greenhouse.

Plant phytotoxicity was rated approximately every week throughout the 38-41 day test period. The phytotoxicity ratings were based on a 0-9 scale, in which 0 indicated no effect and 9 indicated total plant effect or mortality.

Thirty-eight to 41 days after treatment, plants within treatment replicates (pots) were cut at the soil level and dried at 40°C for a minimum of 48 hours.

The mean plant height was determined at test termination. Plant height was measured by extending the seedling to its maximum height and recording the height to the nearest millimeter from the cut base.

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

E. **Statistics:** All data were entered into a spreadsheet which calculated means, standard deviations, and percent inhibition. Treatment means were used to calculate the percent inhibition resulting from the treatment. The percent inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{(\text{sol. control mean} - \text{treat. mean})}{\text{sol. control mean}} \times 100$$

A randomized complete block analysis of variance was performed on treatment level x replicate means. Treatment level means were submitted to a one-tailed Dunnett's test (alpha = 0.05) to determine which treatment concentrations differed from control levels when analysis of variance indicated a significant treatment effect. No-observed-effect concentrations (NOECs) were determined as the highest treatment level not statistically different from the control.

When species were significantly different from the solvent control and demonstrated a dose-responsive pattern with a response greater than 50%, least squares linear regression was performed to determine the EC_{25} and EC_{50} values.

12. **REPORTED RESULTS:**

Phytotoxicity Rating: The phytotoxic effects of clomazone on the test plants were not statistically analyzed. In general, white bleaching was the most prevalent sign of compound damage, which increased with increasing concentration. This bleaching was observed in all control and clomazone treatments with all species tested except soybean. Wilting and necrosis were observed in clomazone-treated plants as well as bleaching. Generally, growth continued and the plants outgrew the symptoms except in the 0.58 and 1.7 lb ai/A treatments.

Shoot Length: By 38-41 days after treatment, cabbage, soybean, and tomato demonstrated no significant reduction in plant height at any rate tested when compared to the solvent controls. The remaining species exhibited reductions in height at some tested rate of clomazone. The NOEC values (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = tomato = cabbage ($\geq$1.7) < cucumber (0.58) < carrot = lettuce = oat = ryegrass (0.19) < corn (0.065) < onion (0.022).

In the case of onion, the treatment means were compared to the negative control rather than the solvent control. This was done because the onion solvent control plant height was determined to be outlying.

Regression analysis could not be conducted on cabbage, carrot, cucumber, soybean, and tomato, due to a lack of dose response. The EC values for the remaining species are presented in Summary Table I (attached).

Shoot Dry Weight: By 38-41 days after treatment, soybean demonstrated no significant reduction in plant dry weight at any rate tested when compared to the solvent controls. The remaining species exhibited reductions in weight at some tested rate of clomazone. The NOEC values (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean ($\geq$1.7) < cucumber (0.58) < tomato (0.19) < cabbage = carrot = corn = oat = ryegrass (0.065) < onion = lettuce (0.022).

In the case of onion, the treatment means were compared to the negative control rather than the solvent control. This was done because the onion solvent control plant weight was determined to be outlying.

Regression analysis could not be conducted on cucumber and soybean due to a lack of dose response. The EC values for the remaining species are presented in Summary Table II (attached).

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

The Quality Assurance Unit was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards. Statements of compliance to GLP and QA were enclosed in the report. The soil analysis was conducted at a laboratory that does not fully comply with GLP standards.

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:

The maximum labeled rate of the compound was not reported.

The rate progression was three-fold rather than two-fold.

The controls were contaminated by the test material.

B. **Statistical Analysis:** Probit and mean comparison (Dunnett's) analyses were conducted on onion dry weight (the most sensitive species) data in comparison to the negative control data. The reviewer's values are in general agreement with the author's (see attached printouts).

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C. **Discussion/Results:** This study is not scientifically sound and does not meet the requirements for a Tier 2 vegetative vigor test using non-target plants. Because the negative and solvent controls were contaminated with the test compound, there was no valid reference to compare plant damage against. Perhaps the study could be blocked by treatment or the controls could be housed in a separate greenhouse due to the high volatility of the test compound.

Plants were dried at 40°C for a minimum of 48 hours to determine dry weight values. To the reviewer, this seems to be a low temperature for drying plant samples. Temperatures of 70-100°C are most often used when drying fresh plant samples.

Phytotoxicity: Although the phytotoxicity data was not statistically analyzed, ratings on the amount of damage were tabularized. From the final rating data (38-41 days after treatment), the reviewer visually determined NOECs. The NOEC values (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean (0.58) < cucumber (0.065) < cabbage = carrot (0.022) < corn = oat = lettuce = ryegrass (0.007) < onion = tomato (<0.007).

In this case, onion and tomato were equally the most sensitive species in terms of phytotoxicity. The lowest-observed-effect concentration (LOEC) would be 0.007 lb ai/A for these two species.

Shoot Length: Onion was the most sensitive species with respect to shoot length. When treatment means were compared to negative control means, the NOEC, LOEC, EC_{25}, and EC_{50} values were 0.022, 0.065, 0.043, and 0.13 lb ai/A, respectively.

Shoot Dry Weight: Onion was again the most sensitive species with respect to shoot dry weight. When treatment means were compared to negative control means, the NOEC, LOEC, EC_{25}, and EC_{50} values were 0.022, 0.065, 0.014, and 0.05 lb ai/A, respectively.

D. **Adequacy of the Study:**

(1) Classification: Invalid.

(2) **Rationale:** Contaminated negative and solvent controls precluded valid results from being drawn.

(3) **Repairability:** No.

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

SUMMARY TABLE I

Results From the Vegetative Vigor Tests with Clomazone

Species	Vegetative Vigor Shoot Length EC_{25} (lb a.i./acre)	Vegetative Vigor Shoot Length EC_{50} (lb a.i./acre)	Vegetative Vigor Shoot Length Observed NOEC (lb a.i./acre)
Cabbage	ND	ND	$\geq$1.7
Carrot	ND	ND	0.19
Corn	0.30	1.1	0.065
Cucumber	ND	ND	0.58
Lettuce	0.23	0.32	0.19
Oat	0.47	1.1	0.19
Onion	0.0040	0.029	ND
*Onion	0.043	0.13	0.022
Perennial Ryegrass	0.72	1.6	0.19
Soybean	ND	ND	$\geq$1.7
Tomato	ND	ND	$\geq$1.7

ND = Not Determined

*Compared to the control blank treatment

SUMMARY TABLE II

Results From the Vegetative Vigor Tests with Clomazone

Species	Vegetative Vigor Shoot Weight EC_{25} (lb a.i./acre)	Vegetative Vigor Shoot Weight EC_{50} (lb a.i./acre)	Observed NOEC (lb a.i./acre)
Cabbage	0.077	0.34	0.065
Carrot	0.19	0.30	0.065
Corn	0.13	0.20	0.065
Cucumber	ND	ND	0.58
Lettuce	0.031	0.092	0.022
Oat	0.16	0.25	0.065
Onion	0.0001	0.0014	ND
*Onion	0.014	0.050	0.022
Perennial Ryegrass	0.14	0.28	0.065
Soybean	ND	ND	$\geq$1.7
Tomato	0.38	1.5	0.19

ND = Not Determined

*Compared to the control blank treatment

onion dry weight

Summary Statistics and ANOVA

Transformation = None

Group (rate (lb ai/A))	n	Mean	s.d.	cv%
1 = control	4	84.5000	10.8474	12.8
2 0.007	4	59.0000	34.4770	58.4
3 0.022	4	67.5000	59.7132	88.5
4* 0.065	4	36.7500	15.2179	41.4
5* 0.19	4	17.0000	7.7889	45.8
6* 0.58	4	7.7500	9.7082	125.3
7* 1.76	4	1.7500	3.5000	200.0

NOEC= ~~0.065~~ 0.022 lb ai/A

LOEC= 0.065 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

Minumum detectable difference for Dunnett's test = -47.537712
This difference corresponds to -56.26 percent of control

Between groups sum of squares = 24541.857143 with 6 degrees of freedom.

Error mean square = 752.964286 with 21 degrees of freedom.

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

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**************************************************
*                                                *
* 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.                           *
*                                                *
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onion dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0010	0.0000	0.0068
EC 5.00	0.0034	0.0000	0.0151
EC10.00	0.0065	0.0001	0.0233
EC15.00	0.0100	0.0002	0.0316
EC50.00	0.0649	0.0139	0.1488
EC85.00	0.4193	0.1782	4.4761
EC90.00	0.6520	0.2541	12.8543
EC95.00	1.2541	0.4102	64.2589
EC99.00	4.2766	0.9437	1403.6559

$y = 6.52 + 1.28(x)$

y = probit % inh. b.t. ond

x = log (rate)

$EC_{25} = 0.019$ lb ai/A