8-29-91

MRID No. 414035-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Facet (BAS 514 00H).
Shaughnessey No. 128947-3.

2. **TEST MATERIAL:** BAS 514 00H (Quinchlorac); Sample No. 150732; 96.5% purity; a white powder.

3. **STUDY TYPE:** Non-Target Plants: Seed Germination/Seedling Emergence Phytotoxicity Test - Tier 2. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, and Onion.

4. **CITATION:** Chetram, R.S. 1989. Tier 2 Seed Germination/ Seedling Emergence Nontarget Phytotoxicity Test BAS 514 00H. Laboratory Project No. LR89-03B. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by BASF Corporation, Research Triangle Park, NC. EPA MRID No. 414035-01.

5. **REVIEWED By:**

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

Signature: Mark Mossler
Date: 4/2/91
Charles Lewis 8/27/91

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 4/2/91

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

Signature: Henry T. Craven
Date: 8/29/91

20 hrs

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7. **CONCLUSIONS:**

Seed Germination: This study meets the requirements for a Tier-2 seed germination test using non-target plants. Treatment of the seeds with BAS 514 00H up to the maximum application rate (2.0 lb ai/A) did not have any significant effect on seed germination of any plant species tested except for lettuce, soybean, and carrot. Carrot was the most sensitive species with NOEC, EC_{25}, and EC_{50} values of 0.125, 0.34, and 0.622 lb ai/A, respectively.

A significant decrease in radicle length was observed for all species except cabbage and ryegrass. Carrot was again the most sensitive species with NOEC, EC_{25}, and EC_{50} values of 0.0125, 0.01, and 0.038 lb ai/A, respectively.

Seedling Emergence: This study meets the requirements for a Tier-2 seedling emergence test using non-target plants. Cucumber was the most sensitive species to BAS 514 00H for all parameters tested except plant height and emergence. In these cases, carrot was the most sensitive species in the emergence test and lettuce and tomato were equally sensitive with respect to plant height.

The NOEC, EC_{25}, and EC_{50} values for the most sensitive species (carrot) for the emergence and survival portion of the study are 0.05, 0.033, and 0.084 lb ai/A, respectively.

The NOEC value for the most sensitive species (cucumber) for the phytotoxicity portion of the test was 0.01 lb ai/A. No EC values were estimated for this section.

The NOEC, EC_{25}, and EC_{50} values for lettuce (one of the most sensitive species with respect to plant height) are 0.0125, 0.03, and 0.096 lb ai/A, respectively. The NOEC, EC_{25}, and EC_{50} values for tomato (the other most sensitive species) are 0.0125, 0.039, and 0.133 lb ai/A, respectively.

The NOEC, EC_{25}, and EC_{50} values for cucumber dry weight (the most sensitive species) are 0.0125, 0.012, and 0.045 lb ai/A, respectively.

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

9. **BACKGROUND:** N/A.

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

11. **MATERIALS AND METHODS:**

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

B. **Test System:**
Seed Germination: Two circles of blue blotter were placed in the bottom of a glass petri plate. The test solutions were prepared with 190 ml of water and 10 ml of acetone and then diluted with water from a well located at the testing facility. Fifteen milliliters of the test solution were added to each plate of soybean, cucumber, oat, and corn. Ten milliliters were added to plates of lettuce, carrot, tomato, cabbage, ryegrass, and onion.

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 same concentration were then randomly placed in plastic boxes (12.25 x 9.0 x 4.1 inches) with sealed lids to prevent moisture loss. The petri plates were incubated in the dark at 25 ±1°C for 7 days, except lettuce, which was incubated at 18 ±1°C.

Seedling Emergence: Ten seeds of each crop were planted in plastic pots (7.5 x 7.5 x 6.0 cm), filled with sterilized soil obtained from the laboratory facility. A plexiglass template was used to create planting holes in the soil, thus allowing for uniform planting depth and seed distribution. An analysis of the soil was provided in the report. Each treatment replicate was placed on an aluminum tray (6.125 x 31.125 cm). The spray plot was 3.21 x 1.67 ft (i.e., 5.36 ft^2).

Soybean and corn were planted at a depth of 2.5 cm, while the remaining eight species were planted at a depth of 1.3 cm. All applications were performed with a belt sprayer equipped with a single nozzle. A nozzle height of 12 inches and a nozzle pressure of 50 psi were used. The test spray solutions were prepared by dissolving BAS 514 00H in water and acetone, then diluting with water from a well located at the testing

facility. 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:** BAS 514 00H was applied at the rates of 0, 0.125, 0.25, 0.5, 1.0, and 2.0 lb ai/A to all plant species. A study continuation was required for soybean, lettuce, carrot, tomato, cucumber, and corn. The dose range was 0, 0.013, 0.025, 0.05, 0.1, and 0.2 lb ai/A. The second continuation study with the dose range of 0, 0.001, 0.003, 0.005, 0.01, and 0.02 lb ai/A was required for lettuce, carrot, tomato, and cucumber. The test solutions were corrected for the percent purity of the test material (96.5%).

D. **Design:**
Seed Germination: Each treatment/crop combination was replicated three times (i.e., 10 seeds/plate, 3 plates/ treatment). After 5 or 7 days of incubation, the seeds were removed from the petri plates and the radicle lengths were measured to the nearest millimeter. Percent seed germination and mean radicle length were calculated for all germinated seeds. Seeds were considered germinated if the radicle was at least 5 mm long.

Seedling Emergence: Each crop/treatment combination was replicated three times (i.e., 10 seeds/pot, 3 pots/ treatment level). After treatment, the pots were randomized within crops and among treatments and placed in an on-site greenhouse.

The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment. Seedling height and phytotoxicity ratings were recorded 7, 14, and 21 days (and 28 days for carrot and lettuce) after treatment for all species. Twenty-one days after treatment (28 days for carrot and lettuce), the plants within treatment replicates (pots) were cut at the soil level and dried in a pre-weighed paper bag at 70°C for a minimum of 48 hours. After drying, the dry weight of the plant material was recorded.

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; and 4-indicates a total effect or plant death.

Temperature, relative humidity, photoperiod, and illuminance 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:

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

An analysis of variance table was constructed using the Lotus 1-2-3 raw data spreadsheet. A one-way analysis of variance (ANOVA) model for data with equal subsamples was used to analyze data from the seed germination (radicle length and percent germination) and seedling emergence (percent emergence) tests. A one-way ANOVA model for data with unequal subsamples was used to analyze the seedling height and weight data. Treatment mean separation was achieved using the Lotus 1-2-3 spreadsheet. Means were separated by Duncan's New Multiple Range Test. The percent detrimental effect values were input into a SAS probit analysis program to calculate EC values.

12. **REPORTED RESULTS:**

Seed Germination: No significant ($p< 0.05$) difference in percent germination existed for any of the test species except for lettuce, soybean, and carrot. The NOEC values (in lb ai/A), for these three species were 0.5, 0.5, and 0.125, respectively. For the remainder of the species, the NOEC value for percent germination was the maximum rate of 2.0 lb ai/A. Therefore, no EC_{25} or EC_{50} values were computed due to the lack of significant rate effects for these seven species. The EC values for soybean, lettuce and carrot are listed in Table 12 (attached).

Cabbage and ryegrass demonstrated no significant difference ($p< 0.05$) in radicle length when compared to the control. The NOEC for these two species was therefore the maximum application rate of 2.0 lb ai/A. The remaining eight

species showed various amounts of radicle length inhibition. The NOEC values (in lb ai/A) for these species, in increasing sensitivity, are:

oat = corn (1.0) < soybean = onion (0.125) < lettuce (0.025) < tomato = cucumber (0.02) < carrot (0.0125).

Since ryegrass and cabbage did not show a dose related response, no EC values could be computed. The EC values for the other eight species are listed in Table 11 (attached).

Seedling Emergence:
Percent Emergence: Carrot was the most sensitive to BAS 514 00H for percent emergence. Lettuce and tomato were also affected by herbicide doses less than the maximum labelled rate. The NOEC values (in lb ai/A) for the ten tested plant species, in increasing sensitivity, are:

soybean = cucumber = cabbage = oat = ryegrass = corn = onion (2.0) < tomato (0.5) < lettuce (0.25) < carrot (0.05).

Cucumber, oat, ryegrass, and onion did not demonstrate a dose response to BAS 514 00H at the concentrations tested, therefore, a probit analysis could not be conducted. Subsequently, EC values were not estimated for these species. The EC values for the remaining species are listed in Table 54 (attached).

Plant Phytotoxicity: Based on 21 day (28 day for carrot and lettuce) phytotoxicity ratings, cucumber was the most sensitive species exposed to the herbicide. The NOEC values (in lb ai/A) for the ten species, in increasing sensitivity, are:

ryegrass (1.0) < onion (0.5) < oat (0.25) < corn (0.2) < soybean = cabbage (0.125) < carrot (0.1) < lettuce = tomato (0.0125) < cucumber (0.01).

No EC values were computed from the phytotoxicity data.

Plant Height: Only cabbage demonstrated no significant difference between control and treated plants for plant height. The resulting NOEC was 2.0 lb ai/A. Tomato and lettuce were equally sensitive to applications of BAS 514 00H with respect to plant height. The NOEC values (in lb ai/A) for the ten species, in increasing sensitivity, are:

cabbage (2.0) < oat = ryegrass (1.0) < corn (0.2) < soybean = onion (0.125) < carrot = cucumber (0.025) < lettuce = tomato (0.0125).

All plant species demonstrated a dose response, and subsequently, EC values could be computed. The EC values for plant height are presented in Table 55 (attached).

Plant Dry Weight: All plant species exhibited significant differences between some rate of BAS 514 00H and the control. Cucumber was the most sensitive species with respect to plant dry weight. Oat and ryegrass were equally the most tolerant species. The dry weight NOECs, (in lb ai/A), in increasing sensitivity to BAS 514 00H are:

oat = ryegrass (1.0) < corn (0.2) < cabbage = onion (0.125) < carrot (0.1) < soybean (0.05) < lettuce = tomato (0.02) < cucumber (0.0125).

All plant species demonstrated a plant dry weight response. The EC values for the ten plant species are listed in Table 56 (attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions 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:

All plants in each replicate were weighed together, then the total weight was divided by the total number of plants to obtain each replicate mean value. The plants should have been individually weighed so the variation among plants within each replicate could be accounted for in the statistical analysis of the data.

B. **Statistical Analysis:** Probit analyses were conducted on carrot (the most sensitive species) radicle length data for the seed germination test and percent emergence for the seedling emergence test. The EC_{25} and EC_{50} values (attached) were slightly higher than those obtained by the author. Dunnett's comparison test was used to predict the NOEC values (attached) from these same data sets. The NOEC is in agreement

with the author's for carrot emergence, but is one dilution greater than those obtained by the author for radicle length. Since the author obtained more conservative estimates for the NOEC, and EC values, these will better protect non-target plants and are accepted as the NOEC and EC values.

C. **Discussion/Results:**

Seed Germination: No significant differences were observed between the control and any rate of BAS 514 00H for percent germination on the ten species except in lettuce, soybean, and carrot. Carrot was the most sensitive to BAS 514 00H with an NOEC value of 0.125 lb ai/A. Soybean and lettuce were equally sensitive with an NOEC value of 0.5 lb ai/A.

Carrot radicle length was most sensitive to BAS 514 00H. The NOEC for carrot radicle length was 0.0125 lb ai/A. All species except cabbage and ryegrass were significantly affected to some degree by application of BAS 514 00H. The most tolerant species were cabbage and ryegrass which had an NOEC value of 2.0 lb ai/A.

Seedling Emergence:
Seedling Emergence: Carrot was the most sensitive species treated with BAS 514 00H with an estimated NOEC value of 0.05 lb ai/A. The most tolerant species were soybean, cucumber, cabbage, oat, ryegrass, corn, and onion. These species all had an NOEC value of 2.0 lb ai/A.

Plant Phytotoxicity: Cucumber was the most sensitive species with regard to plant phytotoxicity. The NOEC for cucumber is 0.01 lb ai/A. The most tolerant species is ryegrass, with an NOEC of 1.0 lb ai/A. Values for EC_{25} and EC_{50} were not constructed.

Plant Height: Cabbage was the only species that was unaffected by BAS 514 00H application at any rate. The NOEC for this plant was 2.0 lb ai/A. Lettuce and tomato were the most sensitive to BAS 514 00H applications. The NOEC value for these two species was 0.0125 lb ai/A.

Plant Dry Weight: Plant dry weight was significantly affected in all species. Cucumber was the most sensitive species with respect to dry weight. The NOEC for this species was 0.0125 lb ai/A. Oat and ryegrass

were the most tolerant species, with a subsequent NOEC value of 1.0 lb ai/A.

These studies are scientifically sound and fulfill the guideline requirements for the Tier 2 seed germination/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.

EFED review dated 8/29/91 (Seedling Emergence - Quinclorac)

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carrot radicle length

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
rate (lb ai/A)				
1 = control	30	25.1667	21.3268	84.7
2 0.0125	30	20.9333	13.3673	63.9
3 0.025	30	19.3333	12.1891	63.0
4* 0.05	30	12.8667	6.4846	50.4
5* 0.1	30	5.8000	5.0678	87.4
6* 0.2	30	3.5000	2.6749	76.4

NOEC = 0.025 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 = -6.881282
This difference corresponds to -27.34 percent of control

Between groups sum of squares = 11334.733333 with 5 degrees of freedom.

Error mean square = 142.830268 with ** 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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carrot radicle length

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0027	0.0014	0.0042
EC 5.00	0.0063	0.0040	0.0088
EC10.00	0.0100	0.0069	0.0132
EC15.00	0.0137	0.0100	0.0174
EC50.00	0.0508	0.0434	0.0594
EC85.00	0.1886	0.1483	0.2597
EC90.00	0.2572	0.1948	0.3746
EC95.00	0.4074	0.2908	0.6471
EC99.00	0.9652	0.6130	1.8150

$Y = 7.35 + 1.82X$ $\quad EC_{25} = 0.022$

Y = % probit inhibition

X = log (rate)

CARROT EMERGENCE

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
rate (lb ai/A)				
1 = control	3	76.6667	5.7735	7.5
2 0.0125	3	86.6667	11.5470	13.3
3 0.025	3	86.6667	15.2753	17.6
4 0.05	3	60.0000	10.0000	16.7
5* 0.1	3	46.6667	15.2753	32.7
6* 0.2	3	10.0000	17.3205	173.2

NOEC = 0.05 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 = -26.787919
This difference corresponds to -34.94 percent of control

Between groups sum of squares = 13111.111111 with 5 degrees of freedom.

Error mean square = 172.222222 with 12 degrees of freedom.

Bartlett's test p-value for equality of variances = .820

CARROT EMERGENCE

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0217	0.0045	0.0378
EC 5.00	0.0342	0.0111	0.0526
EC10.00	0.0436	0.0178	0.0634
EC15.00	0.0514	0.0242	0.0725
EC50.00	0.1025	0.0727	0.1575
EC85.00	0.2047	0.1387	0.5386
EC90.00	0.2411	0.1571	0.7410
EC95.00	0.3073	0.1877	1.1971
EC99.00	0.4841	0.2588	2.9803

$y = 8.41 + 3.45x$

$EC_{25} = 0.066$

y = % probit inhibition

x = log (rate)