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

NOV 6 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

D159521

MEMORANDUM

SUBJECT: Review of Non-target Terrestrial Plant Studies For The Herbicide MSMA - List B, Phase V, Re-Registration Action

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

Douglas J. Urban 11/5/91

TO: Jay Ellenberger, PM - 50
Reregistration Branch
Special Review And Reregistration Division (H7508W)

The Ecological Effects Branch has reviewed the following non-target terrestrial plant phytotoxicity studies submitted in support of MSMA reregistration (Phase V review of List B chemical):

1) 123-1 Seed Germination and Seedling Emergence. MRID No. 417055-01. Chetram, R.S. 1990. Tier 2 Seed Germination/ Seedling Emergence Nontarget Phytotoxicity Study Using MSMA. Lab. Project No. LR90-423. Conducted by Pan Ag. Labs. Inc., Madera, CA. Submitted by Luxembourg Ind. Ltd., Memphis, TN. using 51.0% technical MSMA.

2) 123-1 Vegetative Vigor. MRID No. 417055-02. Chetram. R.S. 1990. Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study Using MSMA. Lab. Project No. LR90-422. Conducted by Pan Ag. Labs. Inc., Madera, CA. Submitted by Luxembourg Ind. Ltd., Memphis, TN. using 51% technical MSMA.

The results of the above listed studies were:

123-1 Seed Germination - CORE

123-1 Seedling Emergence - SUPPLEMENTAL

123-1 Vegetative Vigor - CORE

The seedling ~~e~~emergence study was listed as supplemental because a valid EC25 value could not be determined for cabbage, the most sensitive species tested. The seedling emergence test for cabbage must be repeated using lower dosages.

A terrestrial non-target plant phytotoxicity risk assessment cannot be conducted until after receipt and review of a valid seedling emergence test for cabbage.

An aquatic non-target plant phytotoxicity risk assessment cannot be conducted until after receipt and review of the following outstanding studies:

1.) Lemna gibba* (Aquatic macrophyte)

2.) Selenastrum capricornutum* (Freshwater green algae)

3.) Skeletonema costatum (Marine diatom)

4.) Anabaena flos-aquae (Blue-green algae)

5.) A freshwater diatom (Unspecified species)

If you have any questions regarding this review, please contact Richard Petrie at 557-7358 (Room 1024-G, CM-2).

* These studies are in EEB and currently under review, see DPBarcode D 159519.

cc Amy Rispin

DATA EVALUATION RECORD

NOV 6 1991

1. **CHEMICAL:** MSMA.
Shaughnessey No. 013803.

2. **TEST MATERIAL:** MSMA; Monosodium Methanearsonate; Lot No. 20338-97-38; 51.0% purity; a clear yellow liquid.

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, Onion.

4. **CITATION:** Chetram, R.S. 1990. Tier 2 Seed Germination/ Seedling Emergence Nontarget Phytotoxicity Study Using MSMA. Laboratory Project No. LR90-423. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by Luxembourg Industries (Pamol) Ltd., Memphis, TN. EPA MRID No. 417055-01.

5. **REVIEWED By:**

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

Signature: Mark A. Mossler
Date: 5/7/91

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 5/9/91

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

Signature: Richard C. Petrie 10/08/91
Date: for Daniel Rieder

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7. **CONCLUSIONS:**

Seed Germination: This study is scientifically sound and meets the requirements for a Tier-2 seed germination test using non-target plants. Treatment of the seeds with MSMA up to the maximum application rate (2.24 lb ai/A) did not have a significant effect on percent germination for any plant species tested. A significant decrease was observed in oat, ryegrass, cabbage, and soybean radicle length, with soybean being the most sensitive. The NOEC values for these species were 1.12, 1.12, 0.56, and 0.28 lb ai/A, respectively. The NOEC value for the remaining six species was 2.24 lb ai/A. Because of a lack of rate response above 50% for soybean and cabbage, EC_{50} values could not be estimated. The EC_{25} values for soybean and cabbage were 1.666 and 1.007 lb ai/A, respectively.

Seedling Emergence: This study is scientifically sound but does not meet the requirements for a Tier-2 seedling emergence test using non-target plants. Cabbage was the most sensitive species to MSMA for all parameters tested except percent emergence in which case all species were not affected by the maximum application rate of 2.24 lb ai/A. Table 11 (attached) lists the NOEC and EC values for the various parameters measured in the seedling emergence test. The NOEC for cabbage height and weight could not be determined since all test levels reduced both parameters significantly.

8. **RECOMMENDATIONS:** Tests need to be performed on cabbage with lower rates of MSMA to determine the NOEC values for plant height and dry weight.

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.

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B. **Test System:**

Seed Germination: Two circles of blue blotter were placed in the bottom of a glass petri plate (100 x 15 mm). The test solutions were prepared with well water and then diluted to achieve the lower concentrations. Twelve 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 tightly fitting 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 20 ±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. A description 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., 5.35 ft^2).

Soybean, cucumber, oat, and corn were planted at a depth of 2.5 cm, while the remaining six 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 45 psi were used. The test spray solutions were prepared by dissolving MSMA in well water, then diluted to achieve the lower concentrations. The plants were sprayed at the equivalent of 468 l/ha (50 gpa) of water. Each concentration of MSMA contained 50 ppm Triton X-100.

The pots were watered three times a day and a total of 28-51 ml of water was used to irrigate each pot per day.

C. **Dosage:** MSMA was applied at the rates of 0, 0.14, 0.28, 0.56, 1.12, and 2.24 lb ai/A to all plant

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species. Treatment application rates were adjusted for the percent purity of the test material (51.0%).

D. **Design:**

Seed Germination: Each treatment/crop combination was replicated four times (i.e., 10 seeds/plate, 4 plates/treatment). After 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 four times (i.e., 10 seeds/pot, 4 pots/treatment level). The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment. After treatment, all pots except lettuce and ryegrass were randomized within crops and among treatments and placed in an on-site greenhouse. Lettuce and ryegrass pots were placed in a 25°C room for 48 hours to enhance emergence. The pots were then placed in the greenhouse.

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 10, 14, and 21 days after treatment for all species. Twenty-one days after treatment, plant height was recorded and 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.

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

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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 completely randomized blocked analysis of variance was performed on the treatment level x replicate means. The treatment level means were submitted to a one-tailed Dunnett's multiple comparison test to determine treatment differences from the control level. The statistical no-effect concentration is the highest treatment level not statistically different from the control.

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

12. **REPORTED RESULTS:**

Seed Germination: No significant ($p< 0.05$) difference in percent germination existed between any treatment level and the controls for the ten test species. The NOEC value for percent germination was the maximum rate of 2.24 lb ai/A. Therefore, no EC_{25} or EC_{50} values were computed due to the lack of significant rate effects. Carrot demonstrated a significant difference in percent germination at the 0.56 lb ai/A rate, but not at the 1.12 or 2.24 lb ai/A rates. The NOEC value for carrot was taken to be 2.24 lb ai/A.

Oat, ryegrass, cabbage, and soybean demonstrated significant differences in radicle length at various treatment rates when compared to the controls. The most sensitive species was soybean. The NOEC values (in lb ai/A) for the ten species are:

lettuce = carrot = tomato = cucumber = corn = onion (2.24) < oat = ryegrass (1.12) < cabbage (0.56) < soybean (0.28).

Soybean and cabbage where the only two species that demonstrated a dose response curve. However, the percent effect was not high enough to predict the EC_{50} values from the data. The resulting EC_{25} values for soybean and cabbage were 1.666 and 1.007 lb ai/A, respectively.

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Seedling Emergence:

Percent emergence and survival: Through day 14, percent emergence for all species treated did not differ significantly ($p < 0.05$) from the control. The 2.24 lb ai/A rate was the NOEC value for all species. Due to a lack of dose related responses, no EC values could be estimated.

By the end of the testing period (21 days), only cabbage demonstrated any significant differences in percent emergence. The NOEC values for cabbage and the remaining nine species are 1.12 and 2.24 lb ai/A, respectively.

Due to the lack of a rate response by any of the ten species, EC values could not be determined.

Phytotoxicity rating: Based on 21-day observations, only cabbage demonstrated a significant difference between the control and the three highest rates of MSMA tested. The subsequent NOEC values for cabbage and the remaining nine species are 0.28 and 2.24 lb ai/A, respectively.

Plant height: All species tested, except cabbage and oat, demonstrated no significant decreases in plant height when compared to the controls. Cabbage exhibited a significant difference compared to the control when treated with the lowest rate of MCPA, therefore, the NOEC for cabbage is <0.14 lb ai/A. The NOEC value for oat is 1.12 lb ai/A. The NOEC value for the remaining eight species is 2.24 lb ai/A. Only cabbage demonstrated a dose related response curve. The EC_{25} and EC_{50} values for cabbage are 0.270 and 0.683 lb ai/A, respectively.

Plant dry weight: As with other emergence parameters, cabbage dry weight was most sensitive to MSMA, even at the lowest rate of 0.14 lb ai/A. The NOECs for the tested species in order of increasing sensitivity (in lb ai/A) are:

soybean = carrot = corn = ryegrass = tomato = cucumber = onion (2.24) < oat (1.12) < lettuce (0.56) < cabbage (<0.14).

Lettuce and cabbage were the only two crops that demonstrated dose related responses, therefore, EC values could be computed. However, the lettuce EC_{50} value could not be estimated because the detrimental effects were less than 50%. The resulting EC_{25} value for lettuce is 1.072 lb ai/A. The EC_{25} and EC_{50} values for cabbage are 0.116 and 0.316 lb ai/A.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "A no-effect concentration was achieved for soybean, lettuce, carrot, tomato, cucumber, oat, ryegrass, corn, and onion in every parameter measured. A no-effect concentration was reached for all parameters except plant height and dry weight for cabbage. EC_{25} values were determined for soybean, cabbage (radicle length), and lettuce (dry weight). EC_{25} and EC_{50} values were determined for cabbage (plant height, dry weight)."

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.

The NOEC value was not reached for cabbage height or weight. A further dilution progression is required to obtain these values.

B. **Statistical Analysis:** Probit and Bonferroni's analyses (attached) were conducted on soybean and cabbage (the most sensitive species) data for radicle length and dry weight, respectively. The reviewer's results for the soybean data are slightly greater in concentration than the author's. The NOEC value obtained for cabbage dry weight was in agreement with the author's. However, the EC_{25} and EC_{50} values for cabbage dry weight obtained by the reviewer are slightly lower, with values of 0.088 and 0.254 lb ai/A, respectively. Since these values are more conservative and will better protect non-target plants, they will be taken to be the EC_{25} and EC_{50} values for cabbage dry weight.

C. **Discussion/Results:** The NOEC, EC_{25}, and EC_{50} values for all plant species are listed in Table 11 (attached).

Seed Germination: Treatment of the seeds with MSMA up to the maximum application rate (2.24 lb ai/A) did not have any significant effect on the germination of any plant species tested when compared to the control. Because there was no dose response, the EC_{25} and EC_{50} values could not be determined.

The statistical NOEC for cabbage was 1.12 lb ai/A, but since there was a -26% effect, the NOEC was determined to be 0.56 lb ai/A (Tables 2 & 11 - attached). Soybean was the most sensitive of the species tested with respect to radicle length with an NOEC value of 0.28 lb ai/A. Because of the lack of rate response above 50% detrimental effect, an EC_{50} value could not be calculated. The EC_{25} value for soybean radicle length is 1.666 lb ai/A.

Seedling Emergence:
Percent emergence and survival: At the 14 day observation period, all ten species demonstrated no significant differences in emergence in comparison to the controls. The NOEC value was therefore 2.24 lb ai/A. By 21 days after application, only cabbage demonstrated significant differences in percent emergence at the highest rate of MSMA. The NOEC value is therefore 1.12 lb ai/A. The NOEC value for the remaining nine species is 2.24 lb ai/A. A lack of dose related response precluded EC values from being determined for any species tested.

Phytotoxicity rating: By the end of the study (21 days) the only species that was affected by MSMA was cabbage. The NOEC value was determined to be 0.28 lb ai/A. For the remaining nine species, the NOEC value was 2.24 lb ai/A.

Plant height: Plant height of all species except oat and cabbage was not affected by MSMA application. Cabbage was the most sensitive species with an NOEC value of <0.14 lb ai/A. The NOEC value for oat was 1.12 lb ai/A. The remaining species all had an NOEC value of 2.24 lb ai/A. Cabbage was the only species that exhibited a dose response and the subsequent EC_{25} and EC_{50} values were 0.270 and 0.683 lb ai/A, respectively.

Plant dry weight: It is interesting to note that applications of MSMA increased the dry weight of soybean, cucumber, and tomato from 24-25%. The only

species that demonstrated significant dry weight reductions were oat, lettuce, and cabbage. The NOEC values for these three species are 1.12, 0.56, and <0.14 lb ai/A, respectively. The NOEC value for the other seven species is 2.24 lb ai/A. Lettuce and cabbage both exhibited a dose response, however, the detrimental effect on lettuce was not greater than 50%. Therefore, the EC_{50} value could not be determined for lettuce. The EC_{50} value for cabbage was 0.254 lb ai/A. The EC_{25} values for lettuce and cabbage were 1.072 and 0.088 lb ai/A, respectively.

The seed germination study is scientifically sound and fulfills the guideline requirements for the Tier 2 seed germination test using non-target plants. The seedling emergence test using non-target plants does not fulfill the requirements for this type of test. The results indicate that cabbage should be retested using lower rates of MSMA to determine the NOEC values for height and dry weight.

D. **Adequacy of the Study:**

(1) **Classification:** Core for seed germination and supplemental for seedling emergence.

(2) **Rationale:** The study followed the approved protocol for toxicity tests on seed germination using non-target plants. However, lack of NOEC estimates for height and dry weight on the most sensitive species (cabbage) for the seedling emergence test precludes this study from being placed in the core category.

(3) **Repairability:** Additional tests using lower dose rates should be conducted on cabbage for the seedling emergence test.

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

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Pages 12 through 13 are not included.

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Soybean radicle length
File: soy Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	20353.577	4070.715	3.181
Within (Error)	207	264894.366	1279.683	
Total	212	285247.944		

Critical F value = 2.29 (0.05,5,120)
Since F > Critical F REJECT Ho:All groups equal

Soybean radicle length
File: soy Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	89.794	89.794		
2	0.14	88.917	88.917	0.103	
3	0.28	86.333	86.333	0.405	
4	0.56	73.410	73.410	1.952	
5	1.12	64.486	64.486	2.938	*
6	2.24	70.606	70.606	2.195	

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=120,5)

NOEC = 0.56 lb a.i./A.

Soybean radicle length
File: soy Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	34			
2	0.14	36	20.172	22.5	0.877
3	0.28	36	20.172	22.5	3.461
4	0.56	39	19.792	22.0	16.384
5	1.12	35	20.312	22.6	25.308
6	2.24	33	20.613	23.0	19.188

Soybean radicle length

soybean radicle length

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0762		
EC 5.00	0.2698		
EC10.00	0.5294		
EC15.00	0.8342		
EC50.00	5.7030		
EC85.00	38.9895		
EC90.00	61.4416		
EC95.00	120.5337		
EC99.00	426.5717		

$Y = 4.06 + 1.24X$

Y = probit % inhibition

X = log (rate)

$EC_{25} = 1.65$ lb ai/A.

cabbage dry weight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control (rate (lb ai/A))	4	.6740	.2269	33.7
2* 0.14	4	.3858	.1130	29.3
3* 0.28	4	.3783	.1526	40.3
4* 0.56	4	.1800	.0123	6.8
5* 1.12	4	.0983	.0195	19.8
6* 2.24	4	.0565	.0247	43.7

NOEC = < 0.14 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 = -.207171
This difference corresponds to -30.74 percent of control

Between groups sum of squares = 1.070495 with 5 degrees of freedom.

Error mean square = .014779 with 18 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. *
*
****

cabbage dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0064	0.0020	0.0138
EC 5.00	0.0189	0.0079	0.0337
EC10.00	0.0335	0.0162	0.0545
EC15.00	0.0494	0.0263	0.0755
EC50.00	0.2536	0.1951	0.3123
EC85.00	1.3022	1.0094	1.8507
EC90.00	1.9176	1.4126	2.9722
EC95.00	3.4026	2.3021	6.0540
EC99.00	9.9752	5.6779	23.2970

$\hat{Y} = 5.87 + 1.46X$

Y = % probit inhibition

X = log (rate)

$EC_{25} = 0.0886$ lb ai/A.

DATA EVALUATION RECORD

1. **CHEMICAL:** MSMA.
Shaughnessey No. 013803.

2. **TEST MATERIAL:** MSMA; Monosodium Methanearsonate; Lot No. 20338-97-38; 51.0% purity; a clear yellow liquid.

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

4. **CITATION:** Chetram, R.S. 1990. Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study Using MSMA. Laboratory Project No. LR90-422. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by Luxembourg Industries (Pamol) Ltd., Memphis, TN. EPA MRID No. 417055-02.

5. **REVIEWED By:**

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KBN Engineering and
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Signature: Mark A. Mossler
Date: 5/7/91

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 5/9/91

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

Signature: Richard Petrie
Date: for Donald Rice 10/08/91 10-30-91

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7. **CONCLUSIONS:**

This study is scientifically sound and meets the requirements for a Tier-2 vegetative vigor test using non-target plants. Table 7 (attached) summarizes the NOEC and EC values obtained for each species for the measured parameters.

Cabbage was the most sensitive species with respect to phytotoxicity. The NOEC value is estimated at 0.28 lb ai/A.

Soybean and tomato were equally sensitive to MSMA applications with respect to plant height. The NOEC value for these two species was estimated at 0.28 lb ai/A. Because the rate responses were less than 50%, an EC_{50} could not be computed. The EC_{25} values for soybean and tomato are 1.376 and 1.104 lb ai/A, respectively.

Oat was the most sensitive species with respect to plant dry weight. The NOEC value is estimated at 0.14 lb ai/A. The EC_{25} and EC_{50} values for oat are 0.418, and 1.029 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:**
Seedling Establishment: Seeds of each crop were planted in plastic pots (7.5 x 7.5 x 6.0 cm) and filled with a sterilized soil mix. 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 depth of 2.5 cm, while the remaining six species were planted at a depth of 2.3 cm. An analysis of the soil was provided in the report. After emergence, each pot was thinned to five plants/pot. The ten plant species were allowed to grow for 7-18 days before treatment to allow each species to attain the 1-3 true leaf stage. 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., 5.35 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 45-50 psi were used. The test spray solutions were prepared by dissolving 2.104 g of MSMA in well water. Serial dilutions of the maximum concentration were made to achieve the lower application rates. All concentrations contained approximately 50 ppm Triton X-100 surfactant. 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 24-51 ml of water was used to irrigate each pot per day.

C. **Dosage:** MSMA was applied at a rate of 0.0, 0.14, 0.28, 0.56, 1.12, and 2.24 lb ai/A to all plant species. Treatment application rates were adjusted for the percent purity of the test material (51.0%).

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 crops and among treatments and placed in an on-site greenhouse.

Plant height was measured by extending the seedling to its maximum height and recording the height to the nearest millimeter. The mean plant height was calculated for each treatment. Plant weight was evaluated at 21 days after application.

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; 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 effect resulting from the treatment. The percent effect was calculated using the following equation:

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

Plant heights taken prior to treatment were used as a baseline to calculate the percent effect on growth at the 21 day observation period. The percent increase in height from the 0 day reading was calculated using the following equation:

$$\% \text{ increase} = \frac{(\text{21 day mean} - \text{0 day mean})}{\text{0 day mean}} \times 100$$

The percent effect on growth was calculated for each treatment using the following equation:

$$\% \text{ effect} = \frac{(\text{treat. \% increase} - \text{cont. \% increase})}{\text{control \% increase}} \times 100$$

A completely randomized blocked analysis of variance was performed on the treatment level x replicate means. The treatment level means were submitted to a one-tailed Dunnett's multiple comparison test to determine treatment differences from the control level. The statistical no-effect concentration is the highest treatment level not statistically different from the control.

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

12. **REPORTED RESULTS:**

Phytotoxicity rating: Statistical analysis of the 21 day phytotoxicity ratings shows no significant difference between any treatment level and the control for cucumber, carrot, ryegrass, corn, and onion. Soybean, lettuce, and oat were all significantly affected by MSMA application at the highest rate. Tomato and cabbage were significantly affected at the 1.12 and 0.56 lb ai/A rate of MSMA, respectively. The NOEC values for the ten species (in lb ai/A) in increasing sensitivity to MSMA are:

carrot = cucumber = ryegrass = corn = onion (2.24) < soybean = lettuce = oat (1.12) < tomato (0.56) < cabbage (0.28).

Plant height: Treatment of plants with MSMA at the highest rate of 2.24 lb ai/A did not result in a significant effect on carrot, cucumber, ryegrass, and corn. The remaining six species were significantly affected by MSMA at some application rate. The NOEC values (in lb ai/A) for the ten test species, in increasing sensitivity, are:

carrot = cucumber = ryegrass = corn (2.24) < lettuce (1.12) < cabbage = oat = onion (0.56) < soybean = tomato (0.28).

Due to either a lack of significant rate effects or a true dose response, a probit analysis was not conducted nor EC values calculated for carrot, cucumber, ryegrass, corn, and onion. An EC_{50} value could not be calculated for soybean, lettuce, and tomato because the detrimental effect was less than 50%. The resulting EC values are presented in Table 7 (attached).

Plant dry weight: Statistical analysis of the plant dry weight data show no significant difference between the controls and any treatment level for carrot, ryegrass, and corn. The NOEC values (in lb ai/A) for the ten tested species, in increasing sensitivity, are:

carrot = ryegrass = corn (2.24) < soybean = lettuce = tomato = cucumber = onion (0.56) < cabbage (0.28) < oat (0.14).

Due to either a lack of significant rate effects or a true dose response, a probit analysis was not conducted nor EC values determined on lettuce, carrot, cucumber, cabbage, ryegrass, corn, and onion. Soybean, tomato, and oat demonstrated a plant dry weight dose response, therefore, a probit analysis was conducted. The EC values obtained are listed in Table 7 (attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"A no-effect concentration was achieved for soybean, lettuce, carrot, tomato, cucumber, cabbage, oat, ryegrass, corn, and onion in every parameter measured. EC_{25} and EC_{50} values were determined for cabbage and oat, and EC_{25} values for soybean, lettuce, and tomato (plant height). EC_{25} and EC_{50} values were determined for soybean, oat, and EC_{25} value for tomato (dry weight)."

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc., was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards. Statements of compliance with QA and GLPs were included in the report.

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 and Dunnett's analysis were conducted on oat (the most sensitive species) data for plant dry weight (attached). The results for the EC values are in agreement with the author's. The author obtained a more conservative estimate for the NOEC for oat of 0.14 lb ai/A. This value will better protect non-target plants.

C. **Discussion/Results:**
These studies are scientifically sound, and fulfill the requirements for a Tier 2 vegetative vigor test using non-target plants.

Phytotoxicity rating: Based on 21 day phytotoxicity ratings, carrot, cucumber, ryegrass, corn, and onion were the least sensitive to MSMA application. Cabbage was the most sensitive to MSMA. The NOEC values are listed in Table 7 (attached).

Plant height: Carrot, cucumber, ryegrass, and corn were least sensitive with respect to plant height. Soybean and tomato were equally the most sensitive

species when treated with MSMA. Percent effect ranged from 30 for corn to -171 for cabbage. The NOEC and EC values are listed in Table 7 (attached).

Plant dry weight: Carrot, ryegrass, and corn were the least sensitive species with respect to weight. Oat was the most sensitive. Percent effect ranged from 38 for carrot to -100 for cabbage. The NOEC and EC values are listed in Table 7 (attached).

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

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

Page 25 is not included in this copy.

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sat dryweight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
rate (lbai/A)				
1 = control	4	1.0963	.1181	10.8
2 0.14	4	.9893	.1274	12.9
3 0.28	4	.9185	.0676	7.4
4* 0.56	4	.8588	.0350	4.1
5* 1.12	4	.4680	.1332	28.5
6* 2.24	4	.2838	.1105	38.9

NOEC = 0.28 lbai/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 = -.178643
This difference corresponds to -16.30 percent of control

Between groups sum of squares = 2.048306 with 5 degrees of freedom.

Error mean square = .010989 with 18 degrees of freedom.

Bartlett's test p-value for equality of variances = .412

oat dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0471	0.0250	0.0735
EC 5.00	0.1163	0.0748	0.1597
EC10.00	0.1883	0.1333	0.2428
EC15.00	0.2607	0.1960	0.3237
EC50.00	1.0304	0.8697	1.2550
EC85.00	4.0733	2.9754	6.3122
EC90.00	5.6386	3.9378	9.3503
EC95.00	9.1289	5.9504	16.7760
EC99.00	22.5359	12.8537	50.4240

$y = 4.98 + 1.74x$

y = % probit inhibition

x = log (rate)

$EC_{25} = 0.423$ lb ai/A.