

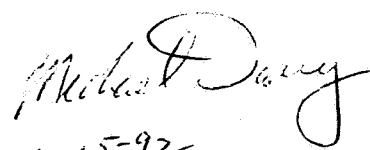
MRID No. 415651-40

DATA EVALUATION RECORD

1. **CHEMICAL:** Acetochlor.
Shaughnessey No. 121601.
2. **TEST MATERIAL:** Formulation WF 2061; 2-chloro-N-ethoxy-methyl-6'-ethylacet-o-toluidide; Preparation reference D3651; 68.6% active ingredient w/w (761.5 g ai/L).
3. **STUDY TYPE:** Non-Target Plants: Seedling Emergence & Vegetative Vigor Phytotoxicity Test - Tier 2. Species Tested: Soybean, Sugar beet, Oilseed rape, Teaweed, Velvetleaf, Pale persicaria, Corn, Winter wheat, Wild oat, Purple nutsedge.
4. **CITATION:** Farmer, D., L. Henderson, P.J.L. Cory, and R.A. Brown. 1990. Acetochlor: A Glasshouse Test to Assess Pre and Post Emergence Effects on Non-Target Plants. Laboratory Project ID 89JH306. Report No. RJ0783B. Conducted by ICI Agrochemicals, Bracknell, Berkshire, UK. Submitted by ICI Americas, Inc., Wilmington, DE. EPA MRID No. 415651-40.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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Date: 1-22-92

7. **CONCLUSIONS:**

The seedling emergence and vegetative vigor study is not scientifically sound and does not fulfill the guideline requirements for a Tier 2 test using non-target plants.

The studies were conducted on only nine species rather than ten, the lower application rates should have been two-fold rather than three- or five-fold, there is probable cause to believe that some of the chemicals may have leached beyond the seeds/nutlets in the seedling emergence study and the vegetative vigor study used two other pesticides to control aphids and mildew without any controls to check the effects that the additional pesticides may have on the plants.

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5. **REVIEWED BY:**

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Date: 2/19/92

7. **CONCLUSIONS:**

The seedling emergence study is not scientifically sound and does not fulfill the guideline requirements for a Tier 2 test using non-target plants. Multiple plants were grown in the same container in this study which led to competition. The vegetative vigor study is scientifically sound but does not fulfill the guideline requirements for a Tier 2 test using non-target plants. Improper rate selection and lack

8. **RECOMMENDATIONS:** Two-fold progressions are required for for all plant studies. Spray solution calculations should be included in the report. The NOEC should be obtained through use of analysis of variance rather than through regression analysis. The seedling emergence and vegetative vigor studies should be conducted with 10 species pre-tested for a minimum of 70 percent germination. Other pesticides should not be used in the study unless there are controls that will show what effects these chemicals have on the plants being studied. It is suggested that bottom-watered be used instead of top-watered when studying seedling emergence.
9. **BACKGROUND:** This study was submitted for an experimental use permit.
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
11. **MATERIALS AND METHODS:**

A. Test Plants:

Dicotyledon plants were represented by six species from five families (i.e., soybean, sugar beet, oilseed rape, teaweed, velvetleaf, and pale persicaria). Monocotyledon plants were represented by four species from two families (i.e., corn, wild oat, winter wheat, and purple nutsedge). Cultivars (where applicable) were provided in the report.

B. Test System:

Seedling Emergence: Each treatment and control replicate consisted of two trays each containing five plant species. One tray contained "warm" climate species and the other contained "cool" climate species. Ten seeds of each crop were planted in plastic seed trays (37 x 23 x 7.5 cm) filled with 5 cm of sandy loam compost. Corn, winter wheat, soybean, oilseed rape, and sugar beet seeds were sown and covered with 1 cm of compost. The remainder of the seeds (nutlets for purple nutsedge) were then sown and covered with an additional 1 cm of compost (i.e., sowing depth of 2 cm for crops, 1 cm for weeds). The compost was not watered prior to spray application so that all species were exposed to the herbicide upon imbibition. After spraying, the seed trays were transferred to a glass-house. The plants were top-watered three times a day and not allowed to become water-logged.

During the experiment, glasshouse conditions for the "warm-climate" species were 18-36°C and 40-88% RH. The

conditions for the "cool-climate" species were 15-38°C and 34-89% RH.

Vegetative Vigor: Velvetleaf, purple nutsedge, oilseed rape, pale persicaria, sugar beet, soybean, and teaweed were initially sown in a peat/perlite mix in seed trays and later transplanted into new 7.5-cm polypropylene pots containing the appropriate compost. The remaining species (i.e., corn, wild oat, and winter wheat) were sown directly into the 7.5-cm pots.

After application, plants were moved to a glasshouse. Pots were watered three times a day and care was taken not to wet the foliage. The glasshouse conditions were the same as in the emergence test.

During the post-emergence test, oilseed rape and sugar beet were fed with the balanced plant food "Vitafeed-S" (N:P:K = 17.5:10:13.5) at 15 and 24 days after chemical application. The "Vitafeed-S" was added (35 ml) to the pots at rates of 1:200 and 1:100 v/v for the two fertilizing periods, respectively. Purple nutsedge was also fertilized at the 24-day time period.

The "cool climate" species in the post-emergence test were treated for mildew and aphids 17 days after test application. The mildew was treated with "Milgo-E" (active ingredient - ethirimol); diluted 6 ml to 1000 ml of water and sprayed to run-off. Aphids were controlled with "Rapid" (active ingredient - pirimicarb).

All applications were made using a hydraulic track-sprayer fitted with a single, even, stainless steel 8001E nozzle. Pressure was measured at 30 psi and the volume of spray was 200 l/ha. The nozzle height was 30 cm for pre-emergence (emergence studies) applications and 35 cm for post-emergence applications (vegetative vigor studies).

The temperature and relative humidity of the glasshouse were maintained at the same conditions as those for the seedling emergence study.

- C. **Dosage:** Acetochlor was applied at the rates of 0, 0.01, 0.03, 0.09, 0.27, 0.81, and 2.52 kg/ha to all plant species for the seedling emergence test and 0, 0.001, 0.004, 0.02, 0.1, 0.5, and 2.52 kg/ha for the vegetative vigor test. Solutions of the highest two rates were made by adding an appropriate volume of

distilled water to a measured amount of the acetochlor formulation. In both pre- and post-emergence tests, lower concentrations were made by serial dilution of the second highest test rate.

D. Design:

Seedling Emergence: Each crop/treatment combination was replicated three times (i.e., 10 seeds/tray, 3 trays/treatment level). Treatments and controls were assessed daily for seedling emergence. The total number of seedlings emerged and number of days to maximum emergence were recorded for each species per replicate. At 7, 14, 21, and 28 days, each replicate tray was observed for plant development and damage of seedlings using a rating scale from 0-100 (Table 15, attached). For each species, each replicate of each rate was observed separately, but the ten (or less) seedlings that had emerged were assessed together to give an overall damage score. Observations of symptomology were also recorded.

At 28 days after application, growth stage for each species in each replicate was recorded according to a growth stage key. The seedlings were then harvested by cutting the stem at soil level; dry weight was assessed as mg per plant by drying each replicate of each species separately to constant weight in an oven at 75°C.

Vegetative Vigor: Each of the 3 replicates in the test consisted of 2 controls and 6 treatments, each having 5 plants (except wild oat, purple nutsedge, and winter wheat, which had 6 plants). The growth stage at application was recorded for each species.

Visual assessments of damage and symptoms in comparison with controls were carried out at 3, 7, 14, 21, and 28 days after application. Growth stage was assessed at 0, 14, and 28 days. After 28 days, plants were harvested at the soil level and dried to constant weight at 75°C. Dry weights were then recorded.

- E. Statistics:** Damage assessments were analyzed using a dose-response of percentage damage on $\log_e$ (application rate). Dose-response curves were linearized by taking the logit transformation percentage damage such that

$$\text{logit (\% damage)} = \log_e \left(\frac{\% \text{ damage} + 0.5}{100.5 - \% \text{ damage}} \right)$$

The slope and intercept of these lines were calculated using simple linear regression of the logit (% damage) on $\log_e$ (rate). $\log_e$ EC₁₀, EC₂₅, and EC₅₀ estimates were then obtained from the line and back-transformed to produce EC₁₀, EC₂₅, and EC₅₀ estimates.

Since the control plants were not scored for damage but used as a reference against which treatment effects were assessed, it is not relevant to calculate a No Observed Effect Level (NOEL) by looking for statistical differences between control and treated plants at low rates of the acetochlor. The concentration which caused 10% damage or less (EC₁₀) was therefore taken as the NOEL and calculated for each species for each date. For this study, non-treatment related plant variation was taken as 10% within the damage scoring system, thus it was proposed that the EC₁₀ represents the level at which no effect, greater than natural variation, would be apparent in the field.

For seedling emergence and final dry weights, values were recorded for control plants. In this case, NOELs were calculated by contrasting control and treated plants. Prior to analysis, seedling count data were transformed using an arc-sine transformation and weight data were transformed using a square-root transformation. A two-way analysis of variance (randomized block design) was performed to obtain an estimate of the within-plot error. This was used in the calculation of Least Significant Difference (LSD) values (comparison of two means) at the 5% and 1% significance levels, which in turn were used to estimate NOELs. The NOEL was taken to be the highest treatment rate, at and below which there was no significant difference from the control.

12. **REPORTED RESULTS:**

Seedling Emergence: No effect concentrations were calculated for each species for percent emergence compared to controls (Table 5, attached). The results showed that NOECs were greater than the highest application rate (2.52 kg/ha) for all species except teaweed, wild oat, and purple nutsedge, which had NOEC values of 0.81, 0.03, and 0.09 kg/ha, respectively. There were no significant treatment effects in the number of days taken for the emergence of each species.

Percent damage was monitored 7, 14, 21, and 28 days after application. The amount of damage increased with time,

however, oilseed rape, teaweed, wild oat, and winter wheat showed some recovery between 21 and 28 days after application (DAA). The 28-day NOECs (Table 6, attached), in increasing sensitivity, for nine test species (in kg/ha) are:

corn (1.29) < winter wheat (0.46) < oilseed rape (0.18) < purple nutsedge (0.13) < sugar beet (0.11) < teaweed (0.10) < wild oat (0.08) < soybean (0.07) < velvetleaf (0.02).

The predominant symptom caused by the pre-emergence acetochlor treatments was overall plant stunt with chlorosis and necrosis on some species. By the end of the study (28 days), only corn had an EC_{25} value greater than 2.52 kg/ha, and corn and soybean had EC_{50} values greater than the maximum rate (2.52 kg/ha). The EC_{25} and EC_{50} values for nine of the ten test species are listed in Tables 7 and 8 (attached).

Table 9 (attached) gives the NOECs of the dry weight measurements taken at 28 days after application. The results from the above ground dry weight measurements demonstrated that 2 species (corn and velvetleaf) had NOECs greater than 2.52 kg/ha. The NOECs for the remaining species in increasing sensitivity are (in kg/ha):

soybean = sugar beet = oilseed rape = winter wheat (0.81) < purple nutsedge = teaweed (0.27) < wild oat (0.03).

Pale persicaria emergence was very poor and subsequently the results for this species were not reported.

Vegetative Vigor: Eight of ten species were affected by post-emergent acetochlor treatments at less than the maximum application rate. Oilseed rape and corn were unaffected by treatments up to the maximum labeled rate. Soybean, sugar beet, oilseed rape, teaweed, winter wheat, and purple nutsedge demonstrated some signs of recovery after 21 days. The 28-day NOEC values (Table 10, attached) were taken to be the EC_{10} values as in the emergence study. These values, in increasing sensitivity, are (in kg/ha):

oilseed rape = corn (>2.52) < sugar beet (2.49) < purple nutsedge (0.92) < soybean (0.86) < winter wheat (0.14) < teaweed (0.05) < velvetleaf (0.03) < pale persicaria (0.02) < wild oat (0.01).

By the end of 28 days, five species sustained greater than 25% damage and three of these sustained damage greater than

50%. Signs of damage were again general plant stunt with necrosis and leaf malformations at the highest test rate on most species. The EC₂₅ and EC₅₀ values at day 28 are listed in Tables 11 and 12 (attached).

Dry weight was unaffected for four species (NOEC = >2.52 kg/ha for soybean, sugar beet, oilseed rape, and purple nutsedge). The NOEC values for the remaining six species (Table 13, attached), in increasing sensitivity are (in kg/ha):

corn = winter wheat (0.5) < velvetleaf (0.1) < teaweed = pale persicaria (0.02) < wild oat (0.001).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The order of increasing sensitivity of the plant species tested to pre-emergence applications of acetochlor, based on 28 DAA EC₁₀ (NOEC) values was corn, winter wheat, oilseed rape, purple nutsedge, sugar beet, teaweed, wild oat, soybean, and velvetleaf. The sensitivities changed somewhat based on EC₂₅ and EC₅₀ values because of sustained damage by some species and recovery by others.

Post-emergent applications of acetochlor up to the highest rate did not affect corn or oilseed rape. Wild oat and winter wheat showed greater sensitivity to post-emergence applications. The sensitivity series was however similar to pre-emergence application.

A quality assurance statement was included in the report, but the GLP compliance page stated that the document was not subject to the requirements of 40 CFR Part 160.

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

A. Test Procedure: The seedling emergence and vegetative vigor studies followed SEP and subdivision J guidelines except for the following:

Pale persicaria had poor emergence (i.e., < 70% for the controls). Therefore, there were only nine species tested for the emergence study. Guidelines require that 10 species be tested.

No solution or spray calculations were included in the report.

No raw data was available for seedling emergence or vegetative vigor study.

The NOEC values given in the report are EC₁₀ values. Using EC₁₀ values as NOEC values is not considered acceptable by the EPA.

The lower rates for the emergence test are three-fold dilutions from the next highest rate. The lower rates for the vegetative vigor test are five-fold dilutions from the next highest rate. Subdivision J and SEP guidelines require that the dilutions be no more than two-fold.

The species were unnecessarily divided into "cool climate" and "warm climate" species and tested in different greenhouses with different climate conditions. It is more preferred to have all of these species grown under same conditions. The variety of soybean 'Amsoy' was placed in the "warm climate" even though the variety is adapted to cooler regions such as Minnesota.

The "cool climate" species in the post-emergence test were treated for mildew and aphids 17 days after test application. The mildew was treated with "Milgo-E" (active ingredient - ethirimol) and aphids were controlled with "Rapid" (active ingredient - pirimicarb). No controlled checks were used to determine what effect the ethirimol and pirimicarb has on the plants.

The seeds and nutlets were watered 3 times daily from the top during the seedling emergence test. Acetochlor is moderately to highly mobile in soil as determined in soil absorption and column leaching studies. It appears that some of the acetochlor may have leached pass the seeds and nutlets thereby minimal amounts may have been available to the seeds and nutlets. It would be more appropriate to have the seeds and nutlets bottom-watered.

It is unclear as to how many seeds/plants were in each replicate per specie. Therefore it becomes unclear if there were sufficient populations for the study. SEP recommends a minimum of 3 replicates of 5 plants per each replicate per specie in each dose for vegetative vigor and a minimum of 3 replicates with 10 seeds per specie in each dose for the seedling emergence study.

B. Statistical Analysis: No statistical analysis was conducted on the seedling emergence and vegetative vigor studies since the studies are invalid studies.

C. Discussion/Results:

Seedling Emergence: The study was conducted in a compost (organic matter= 3.8%). After the seeds/nutlets were planted, the seed trays were watered from the top 3 times a day. It is known that the seed coat imbibes water and not pesticide chemicals. Acetochlor is found to be moderately to highly mobile in soil adsorption and column leaching studies. It can be concluded that an unknown portion of acetochlor may have leached beyond the planting depth and thus affect the results of the study. Therefore the integrity of the results of the study is in doubt. It would be better to bottom-watered the seeds/nutlets.

Vegetative Vigor: The "cool climate" species in the post-emergence test were treated for mildew and aphids 17 days after test application. The mildew was treated with "Milgo-E" (active ingredient - ethirimol) and aphids were controlled with "Rapid" (active ingredient - pirimicarb). No controlled checks were used to determine what effect the ethirimol and pirimicarb has on the plants. Therefore it becomes unclear as to what effect acetochlor has since the other chemicals may play a part in the effects on the plants.

In addition to the above factors for the vegetative vigor and seedling emergence studies, the lower rates for the emergence test are three-fold dilutions from the next highest rate. The lower rates for the vegetative vigor test are five-fold dilutions from the next highest rate. Subdivision J and SEP guidelines require that the dilutions be no more than two-fold.

The SEP recommends at least 10 species be in the study. Only 9 species could be used since pale persicaria (Polygonum lapathifolium) had insufficient germination to be able to be used.

All of the factors cited above concludes that the seedling emergence and vegetative vigor study are not scientifically sound and does not fulfill the guideline requirements for a Tier 2 test for non-target plants.

D. Adequacy of the Study:

- (1) **Classification:** Seedling emergence - Invalid.
Vegetative vigor - Invalid.
- (2) **Rationale:** The studies were conducted on only nine species rather than ten, the lower application rates should have been two-fold rather than three- or five-fold, there is probable cause to believe that some of the chemicals may have leached beyond the seeds/nutlets in the seedling emergence study and the vegetative vigor study used two other pesticides to control aphids and mildew without any controls to check the effects that the additional pesticides may have on the plants.
- (3) **Repairability:** Seedling Emergence - No.
Vegetative vigor - No.

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

ACETOCHLOR

Page _____ is not included in this copy.

Pages 12 through 23 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
 - ☐ Identity of product impurities.
 - ☐ Description of the product manufacturing process.
 - ☐ Description of quality control procedures.
 - ☐ Identity of the source of product ingredients.
 - ☐ Sales or other commercial/financial information.
 - ☐ A draft product label.
 - ☐ The product confidential statement of formula.
 - ☐ Information about a pending registration action.
 - ☒ FIFRA registration data.
 - ☐ The document is a duplicate of page(s) _____.
 - ☐ The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

wild oat dry weight - emergence

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
<i>rate (kg/ha)</i>				
1 = control	6	155.3333	33.6848	21.7
2 0.01	3	142.0000	15.1327	10.7
3 0.03	3	160.3333	43.5469	27.2
4*0.09	3	83.0000	13.2288	15.9
5*0.27	3	41.3333	7.5056	18.2
6*0.81	3	.0000	.0000	.0
7*2.52	3	.0000	.0000	.0

NOEC = 0.03 kg/ha

raw data in Table 25 (attached)

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

Minimum detectable difference for

t-tests with Bonferroni adjustment = -37.897350

This difference corresponds to -24.40 percent of control

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

Between groups sum of squares = 104302.666667 with 6 degrees of freedom.

Error mean square = 610.980392 with 17 degrees of freedom.

 *
 * Warning - the test for equality of variances
 * could not be computed as 1 or more of the
 * variances is zero.
 *

EPA PROBIT ANALYSIS PROGRAM
 USED FOR CALCULATING EC VALUES
 Version 1.4

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.0100	100	8	0.0800	0.0800	0.0152
0.0300	100	0	0.0000	0.0000	0.1197
0.0900	100	46	0.4600	0.4600	0.4258
0.2700	100	74	0.7400	0.7400	0.7888
0.8100	100	100	1.0000	1.0000	0.9634
2.5200	100	100	1.0000	1.0000	0.9975

Chi - Square Heterogeneity = 47.728

```
*****
*                               *
*          WARNING              *
*                               *
* Significant heterogeneity exists. The results reported *
* for this data set may not be valid. The results should *
* be interpreted with appropriate caution.                *
*****
```

Mu = -0.955547
 Sigma = 0.482320

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	6.981147	0.534798	(5.496548,	8.465745)
Slope	2.073311	0.494150	(0.701552,	3.445071)

Theoretical Spontaneous Response Rate = 0.0000

Estimated EC Values and Confidence Limits

Point	Conc.	95% Confidence Limits	
		Lower	Upper
EC 1.00	0.0084	0.0000	0.0281
EC 5.00	0.0178	0.0004	0.0473
EC10.00	0.0267	0.0012	0.0640
EC15.00	0.0350	0.0025	0.0798
EC50.00	0.1108	0.0382	0.3148
EC85.00	0.3502	0.1545	4.6661
EC90.00	0.4598	0.1930	9.8390
EC95.00	0.6884	0.2614	30.5171
EC99.00	1.4671	0.4410	266.9103

EC₁₅ = 0.052 kg/ha

wild oat dry weight - vegetative

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
rate (kg/ha)				
1 = control	6	1123.8333	171.1823	15.2
2 0.001	3	995.0000	219.1187	22.0
3 0.004	3	810.0000	114.9087	14.2
4 0.02	3	968.0000	84.8764	8.8
5 0.1	3	835.3333	31.8957	3.8
6 0.5	3	578.0000	115.0522	19.9
7 2.52	3	187.6667	45.6216	24.3

NOEC = 0.001 kg/ha
RAW data in Table 36 (attached)

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

Minimum detectable difference for
t-tests with Bonferroni adjustment = -209.042704
This difference corresponds to -18.60 percent of control

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

Between groups sum of squares = 2086282.791667 with 6 degrees of freedom.
Error mean square = 18590.009804 with 17 degrees of freedom.
Bartlett's test p-value for equality of variances = .240

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.0010	100	11	0.1100	0.1100	0.0888
0.0040	100	28	0.2800	0.2800	0.1533
0.0200	100	14	0.1400	0.1400	0.2597
0.1000	100	26	0.2600	0.2600	0.3951
0.5000	100	49	0.4900	0.4900	0.5447
2.5000	100	83	0.8300	0.8300	0.6881

Chi - Square Heterogeneity = 38.607

```
*****
*                               *
*          WARNING              *
*                               *
*  Significant heterogeneity exists. The results reported *
*  for this data set may not be valid. The results should *
*  be interpreted with appropriate caution.                *
*****
```

Mu = -0.508382
Sigma = 1.848169

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	5.275073	0.250035	(4.580975,	5.969171)
Slope	0.541076	0.159503	(0.098297,	0.983855)

Theoretical Spontaneous Response Rate = 0.0000

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper
EC 1.00	0.0000	0.0000	0.0010
EC 5.00	0.0003	0.0000	0.0056
EC10.00	0.0013	0.0000	0.0154
EC15.00	0.0038	0.0000	0.0334
EC50.00	0.3102	0.0356	791.3898
EC85.00	25.5306	1.1968	%9301673400000.0000
EC90.00	72.4823	2.2697	% 271852800.0000E+07
EC95.00	340.1510	5.6886	% 126038711.0000E+11
EC99.00	6180.3730	30.3273	% 100000002.0000E+12

NOTE - Upper limits greater than or equal to 1.E20 are really infinite

$$y = 5.28 + 0.54(x)$$

$$y = \text{probit } \% \text{ inhibition}$$

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

$$EC_{50} = 0.017 \text{ kg/ha}$$